Analysis of 28 Arcobacter genomes belonging to different species

Alba, Pérez-Cataluña ; Leekitcharoenphon, Pimlapas; Hendriksen, Rene S.; Aarestrup, Frank Møller; José, Figueras Maria

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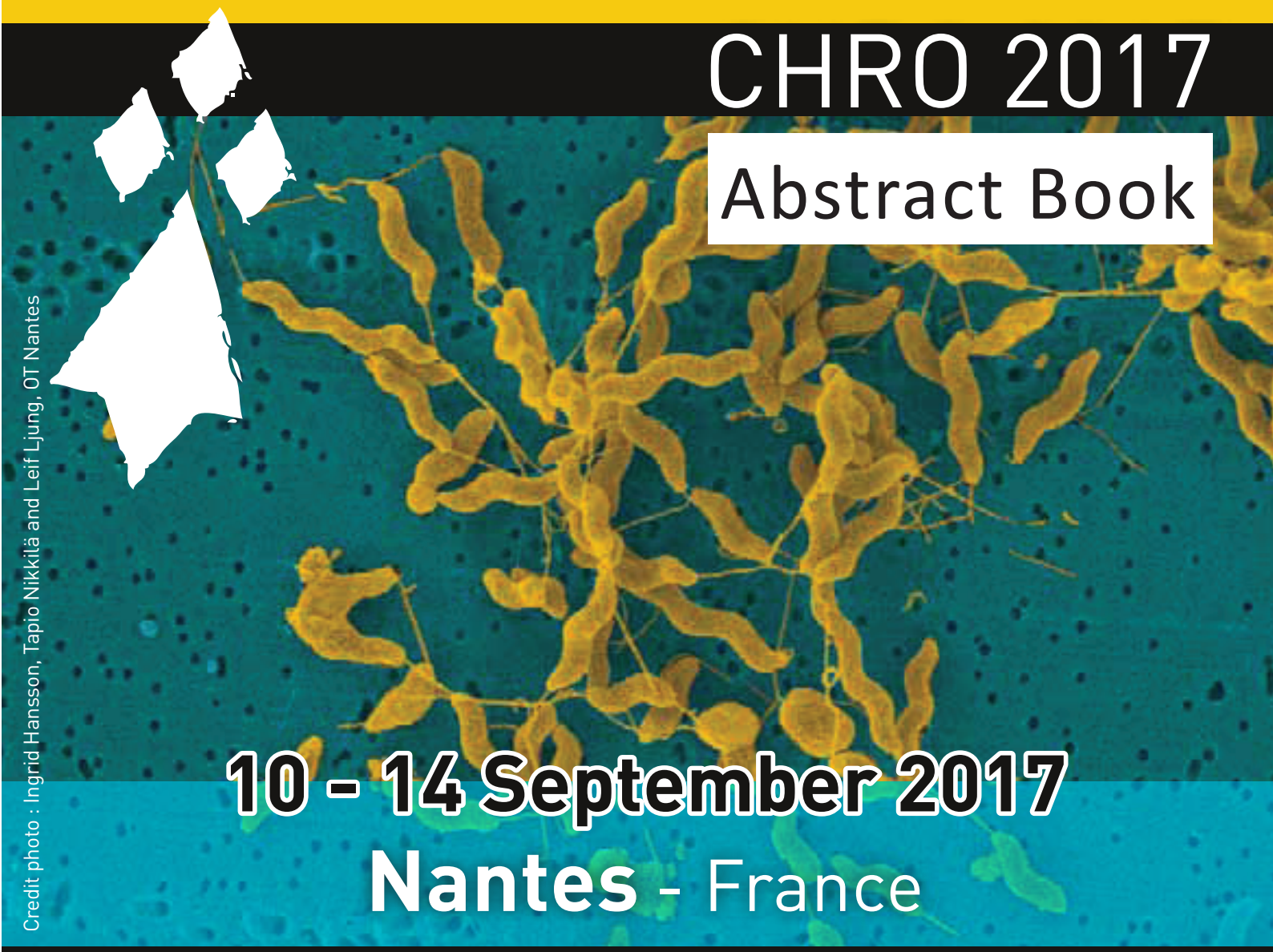

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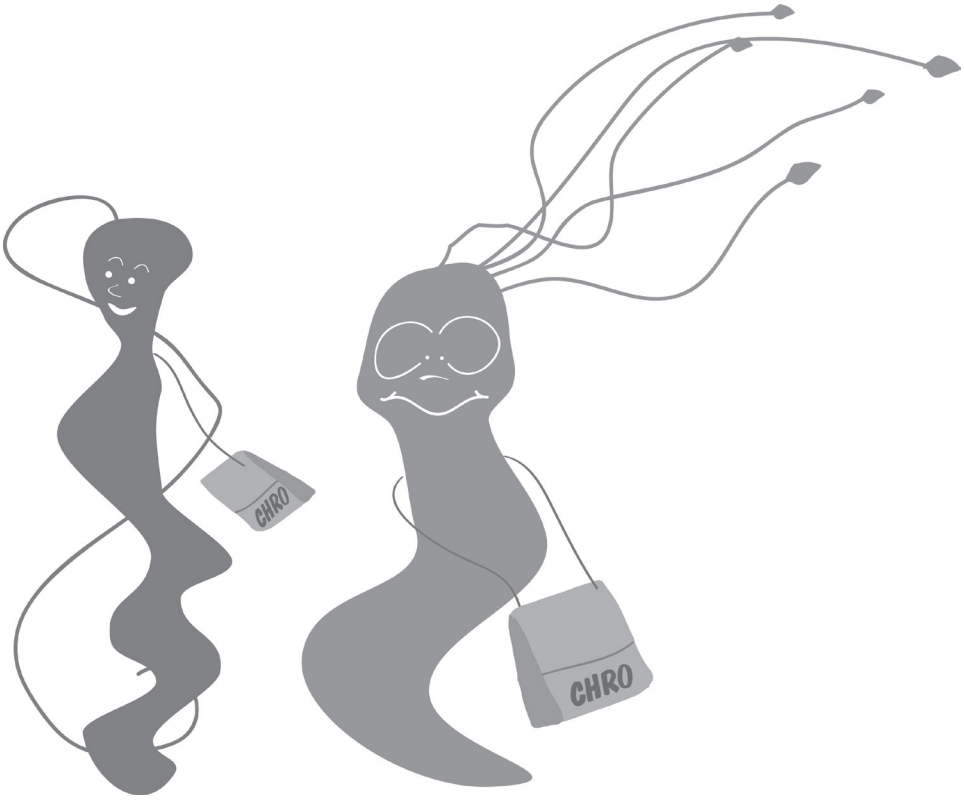

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Table of Contents

ORAL COMMUNICATIONS

Plenary session « Genomic and Evolution 1 »

Chairpersons: SHEPPARD Sam, *United Kingdom* and DE REUSE Hilde, *France*

Oral communications:

Genome-wide identification of cancer associated genetic elements in *Helicobacter pylori*
BERTHENET Elvire, United Kingdom *35*

Genomic and population structure analysis of *Helicobacter pylori* in China reflect geography specific lineages corresponding to historic East Asian migrations
ZHANG Jianzhong, China *36*

Putting bacterial GWAS to the test: can we find the basis of host-association in *Campylobacter jejuni*?
JOHANSSON Håkan, Sweden *37*

Whole genome sequencing of *Campylobacter jejuni* offers an opportunity for in depth investigation into phase variable genes
VRANCKX Katleen, Belgium *38*

Morphological transformation of *Helicobacter pylori* upon expression of a small peptide by a type I toxin-antitoxin system
DE REUSE Hilde, France *39*

Genome and methylome variability of cag pathogenicity island-carrying *Helicobacter pylori* during early human infection
SUERBAUM Sebastian, Germany *40*

Parallel session « Immunology and Host response 1 »

Chairpersons: ETHELBERG Steen, *Denmark* and FERRERO Richard, *Australia*

Oral communications:

Regulation of the LATS2/YAP/TAZ pathway during *Helicobacter pylori* induced gastric carcinogenesis
VARON Christine, France *42*

Interleukin-18 mediates immune responses in *Campylobacter jejuni* infected gnotobiotic mice
HEIMESAAT Markus, Germany *43*

Campylobacter jejuni induces autoimmune peripheral neuropathy *via* Siglec-1 and IL-4 axes in a mouse model of Guillain Barré Syndrome
MANSFIELD Linda, USA *44*

Downregulation of USF1 transcription factors impacts p53 during *Helicobacter pylori* infection and exacerbates gastric carcinogenesis
TOUATI Eliette, France *45*

Parallel session « Methods for Detection, Identification and Characterisation »

Chairpersons: TABODOA Eduardo, *Canada,* RIVOAL Katell, *France* and BESSEDE Emilie, *France*

Oral communications:

A core genome multi-locus sequence typing scheme for stable, comparative analyses of *Campylobacter jejuni* and *C. coli* human disease isolates
CODY Alison, United Kingdom *47*

Comparison of nine selective agars for the detection of *Campylobacter sp.*
REPERANT Elisabeth, France *48*

Evaluation of rapid immunochromatographic test detecting *Campylobacter* in human stools
BESSEDE Emilie, France *49*

Detection of *Campylobacter* in water
FERRARI Sevinc, Sweden *50*

Comparison of testing methodologies for enumerating levels of *Campylobacter* species from fresh whole raw chicken carcasses
JORGENSEN Frida, United Kingdom.....*51*

Parallel session « Poultry and non-poultry epidemiology and ecology of *Campylobacter sp.* »

Chairpersons: DE ZUTTER Lieven, *Belgium* and DENIS Martine, *France*

Oral communications:

Studying *Campylobacter jejuni* in the chicken reservoir
GARAFOLO Giuliano, Italy.....*53*

Molecular characterization of *Campylobacter* from the Canadian Poultry Baseline Survey
TABODOA Eduardo, Canada.....*54*

Characterization of *C. jejuni* isolates from game birds in Finland
KOVANEN Sara, Finland.....*55*

Diversity of *Campylobacter lari* in a One-Health context: Focus on shellfish, wild birds, surface water and human health risk
PENNY Christian, Luxembourg.....*56*

A large survey on *Campylobacter* contamination in bovine production in France
RIVOAL Katell, France.....*57*

Parallel session « Antibiotics and antimicrobial resistance »

Chairpersons: LEHOURS Philippe, *France* and KEMPF Isabelle, *France*

Oral communications:

ST5136 is the largest vertically expanding UK wide clone of *Campylobacter jejuni*
LOPES Bruno, United Kingdom.....*59*

Emerging antibiotic resistance in ruminant *Campylobacter* in the United States
ZHANG Qijing, USA.....*60*

Antimicrobial resistance in *Helicobacter pylori* – a 7 year perspective from Gastrointestinal Bacteria Reference Unit (GBRU), Public Health England
GODBOLE Gauri, United Kingdom *61*

Targeting *Campylobacter jejuni* lytic transglycosylase for drug discovery
LIN June, USA *62*

Plenary session « Pathogenicity and virulence factors »

Chairpersons: KOROLIK Victoria, *Australia* and MEGRAUD Francis, *France*

Oral communications:

Nuclear remodeling in response to the cytolethal distending toxin: formation of nucleoplasmic reticulum
MENARD Armelle, France *64*

Investigating *Campylobacter jejuni* interactions with endoplasmic reticulum in intestinal epithelial cells resulting in induction of the unfolded protein response
DI SARO Gianna, Italy *65*

Identifying virulence factors that lead to abortion from zoonotic transmission and whole genome sequencing
WEIMER Bart, USA *66*

Insights into *Campylobacter jejuni* colonization and enteritis using a novel infant rabbit model
REN Fangzhe, China *67*

Gastric cancer-associated polymorphisms in the *Helicobacter pylori* type IV secretion system protein CagL: a potential biomarker for cancer risk
GORREL Rebecca, Australia *68*

Helicobacter pylori expands and functionally activates a novel gastric stem cell population in a cag pathogenicity island-dependent manner
WROBLEWSKI Lydia, USA *69*

Parallel session « Control strategies for *Campylobacter sp.* »

Chairpersons: HOFSHAGEN Merete, *Norway*, VAN DER LOGT Peter, *New Zealand* and CHEMALY Marianne, *France*

Oral communications:

Relation between soiling of broilers and *Campylobacter* levels during the slaughtering process
KOENE Miriam, The Netherlands.....*71*

Isolation and development of *Campylobacter*-specific bacteriophages to combat bacterial colonization of animal hosts
JOHNSON Tylor, USA.....*72*

A *Campylobacter* putative outer membrane protein induces protective immune responses against an experimental campylobacter infection in broilers
DORY Daniel, France.....*73*

Identification of potential Twin Arginine Translocation (TAT) pathways inhibitors to control *Campylobacter* in poultry
DEBLAIS Loic, USA.....*74*

The in vitro and in vivo effect of Carvacrol in preventing *Campylobacter* infection, colonisation and improve chicken broilers productivity
KELLY Carmel, United Kingdom.....*75*

Parallel session « Pathogenicity and virulence factors 2 »

Chairpersons: GAYNOR Erin, *Canada* and MEGRAUD Francis, *France*

Oral communications:

The Ornithine Decarboxylase inhibitor Difluoromethylornithine effectively reduces *Helicobacter pylori* virulence and induction of inflammation and carcinogenesis
SIERRA Carolina, USA.....*77*

Further characterization of the ligand-binding properties of *Helicobacter pylori* sialic acid binding adhesin A (SabA)
CHANDRAPALA Dilini, Australia.....*78*

Investigating the biogenesis of *Campylobacter jejuni* outer membrane vesicle production
DAVIES Cadi, United Kingdom *79*

Exploring the role of *Campylobacter coli* GT42 enzymes on lipooligosaccharides biosynthesis
ROSSI Mirko, Finland *80*

TNFα affects the internalization of *Campylobacter jejuni* into human intestinal cells
CAPPELIER Jean-Michel, France *81*

Plenary session « Adaptation of *Campylobacter sp*. to environmental conditions 1 »

Chairpersons: KELLY Dave, *United Kingdom*, HADDAD Nabila, *France* and Odile TRESSE, *France*

Oral communications:

Poultry intestinal mucus modulates *Campylobacter jejuni* gene expression
LOOFT Torey, USA *83*

RNAseq reveals complex response of *Campylobacter jejuni* to bile and the ovine gallbladder environment
KREUDER Amanda, USA *84*

Contributions of Phase Variation to Phage-Escape by *Campylobacter jejuni*
BAYLISS Chris, United kingdom *85*

The phospholipidome of *C. jejuni*
WOSTEN Marc, The Netherlands *86*

Membrane protein complexome of *C. jejuni* using 2D blue native/SDS-PAGE
GUERIN Alizée, France *87*

Global control of *Campylobacter jejuni* biology by protein lysine acetylation
PUTTICK Thomas, United Kingdom *88*

Parallel session « Adaptation of *Campylobacter* spp. to environmental conditions 2 »

Chairpersons: KELLY Dave, *United Kingdom* and Odile TRESSE, *France*

The periplasmic methionine sulfoxide reductase system of *Campylobacter jejuni*: A new player in oxidative stress defence
TAYLOR Aidan, United Kingdom *90*

The *Campylobacter jejuni* oxidative stress regulator RrpB is associated with a genomic hypervariable region and altered oxidative stress resistance
GUNDOGDU Ozan, United Kingdom *91*

Campylobacter jejuni FliW modulates CsrA regulation of flagellar and non-flagellar protein expression via protein-protein interaction
FULMER Claudia, USA *92*

Differential survival of hyper-aerotolerant *Campylobacter jejuni* under different gas conditions
JEON Byeonghwa, Canada *93*

Characterisation of aerotolerant forms of a robust chicken colonizing *Campylobacter coli*
CONNERTON Pippa, United Kingdom *94*

Genomic and phenotypic characterization of *C. jejuni* water isolates
NILSSON Anna, Sweden *95*

Parallel session «Immunology and Host response 2»

Chairpersons: ETHELBERG Steen, *Denmark* and FERRERO Richard, *Australia*

Oral communications:

Differential gene expression, signaling pathways, and upstream regulator analysis of acute *Campylobacter jejuni* enteritis in human colon mucosa
BUCKER Roland, Germany *97*

Campylobacter jejuni infection of secondary abiotic mice lacking Nucleotide-Oligomerization-Domain-2
HEIMESAAT Markus, Germany *98*

The role of Nod1 in targeting the immune response towards *Helicobacter pylori* in a genetic model of gastric carcinogenesis
SUAREZ Giovanni, United Kingdom *99*

Helicobacter pylori modulates host cell responses by CagT4SS-dependent translocation of an intermediate metabolite of inner core LPS biosynthesis
JOSENHANS Christine, Germany *100*

The role of gelatinases in *Campylobacter jejuni* infection of gnotobiotic mice
HEIMESAAT Markus, Germany *101*

TRPM2 ion channel modulates macrophage function impacting gastric inflammation during *Helicobacter pylori* infection
ALGOOD Holly, United Kingdom *102*

Plenary session « Emerging and Related Organisms species »

Chairpersons: HOUF Kurt, *Belgium* and CAPPELIER Jean Michel, *France*

Keynote: Evolutionary genomics of gastric *Helicobacter* species revealed interspecies admixture and recombination and evidence for niche adaptation
SMET Annemieke, Belgium *104*

Oral communications:

The importance of *C. rectus* CiaB to host cell invasion and host response
THREADGILL Deborah, USA *105*

Genome analysis reveals novel mobile genetic elements containing virulence-factor-like proteins in *Campylobacter concisus* strains isolated from patients with IBD
ZHANG Li, Australia *106*

Identification of a novel *Campylobacter* species in the gut microbiome of harbour seal pups (*Phoca vitulina*): *Campylobacter blaseri* sp. nov.
ZOMER Aldert, The Netherlands *107*

Arcobacter: novel taxa in New Zealand shellfish
ON Stephen, New Zealand *108*

Isolation and characterisation of a new species of Campylobacter, *C. hepaticus*, responsible for Spotty Liver Disease in chickens
MOORE Robert J., Australia *109*

The immunopathogenic potential of *Arcobacter butzleri* – pathogen or commensal?
HEIMESAAT Markus, Germany *110*

Parallel session « Genomic and Evolution 2 »

Chairpersons: SHEPPARD Sam, *United Kingdom* and DE REUSE Hilde, *France*

Oral communications:

Identification of genomic changes associated with invasiveness in *Campylobacter jejuni*
BIGGS Patrick, New Zealand *112*

Genome-wide introgression and epistasis in agricultural *Campylobacter coli*
MERIC Guillaume, United Kingdom *113*

Whole genome comparisons of *Campylobacter fetus* show benefit of WGS analysis for diagnostics
VAN DER GRAAF Linda, The Netherlands *114*

Recombination-driven adaptation of *Campylobacter jejuni* in cattle
MOURKAS Evangelos, United Kingdom *115*

Parallel session «WGS and outbreak investigation»

Chairpersons: TAUXE Robert, *USA* and VAN CAUTEREN Dieter, *France*

Oral communications:

Using genomics to investigate historic outbreaks of campylobacteriosis in the United Kingdom
BARKER Clare, United Kingdom *117*

A human *Campylobacter fetus* outbreak identified through next generation sequencing
DIUM Brigitta, The Netherlands *118*

Whole-genome sequencing of *Campylobacter jejuni* isolates from patients reveals numerous genetic clusters and possible epidemiologically linked cases
NIELSEN Eva Møller, Denmark *119*

Swedish winter peaks of *Campylobacter* in humans and chicken are connected
SKARIN Hanna, Sweden *120*

Ad hoc whole-genome MLST (wgMLST) analysis reveals multiple *Campylobacter jejuni* clones caused a massive waterborne outbreak in New Zealand in 2016
ZHANG Ji, New Zealand *121*

Comparing the genomic epidemiology of two 'slow-burn' epidemics of *Campylobacter jejuni* in New Zealand
FRENCH Nigel, New Zealand *122*

Parallel session «Tutti Campy»

Chairpersons: HADDAD Nabila, *France* and FEDERIGHI Michel, *France*

Oral communications:

Caecal microbiome modifications across age in a free-range poultry flock naturally-infected with *Campylobacter*
OCEJO Medelin, Spain *124*

Comparing Québec *Campylobacter jejuni* from organically and conventionally raised chickens by whole genome sequencing: nothing special to be signalled.
THIBODEAU Alexandre, Canada *125*

Transcriptomic and functional evaluation of the small RNA CjNC110 in *Campylobacter jejuni*
KREUDER Amanda, USA *126*

The effect of the timing of exposure to *Campylobacter jejuni* on the microbiome and inflammatory responses of broiler chickens
CONNERTON Pippa, United Kingdom *127*

The river runs through: Understanding the human health risks of *Campylobacter jejuni* and *Campylobacter coli* in recreational waters.
BULACH Dieter, Australia *128*

Developing a comparative conditional incidence to analyze relationships between local weather and *Campylobacter* infections in England and Wales
NICHOLS Gordon, United Kingdom *129*

Plenary session « Epidemiology and Public Health »

Chairpersons: TAUXE Robert, *USA* and VAN CAUTEREN Dieter, *France*

Oral communications:

Significant Implication of Ruminants in Human campylobacteriosis in France: Three Genotyping Methods for a Same Trend in Source Attribution?
THEPAULT Amandine, France *131*

How results of campylobacteriosis case-control/case-case studies vary depending on their design?
RAVEL Andre, Canada *132*

Infection pressure by *Campylobacter* and associated risk factors: a seroepidemiological study in the Netherlands
MUGHINI-GRAS Lapo, The Netherlands *133*

Changing diagnostic testing practices and impact on incidence of *Campylobacter* infection– Foodborne Diseases Active Surveillance Network, USA, 2012-2016
GEISSLER Aimee, USA *134*

Risk factors for sporadic *Campylobacter* infections in Denmark: a case-control study in children and young adults
ETHELBERG Steen, Denmark *135*

A combined case-control and molecular source attribution study of human *Campylobacter* infections in Germany, 2011-2014
ROSNER Bettina, Germany *136*

POSTERS

Session « Genomic and Evolution »

How open is the *Campylobacter* genome?
WILKINSON David, New Zealand *138*

The genetic diversity and evolution of plasmids from *Campylobacter jejuni* and *Campylobacter coli* from the United Kingdom (UK)
JANSEN VAN RENSBURG Melissa J., United Kingdom *139*

Campylobacter Strain Diversity – *Campylobacter* is dead; long live *Campylobacter*
FORBES Ken J., United Kingdom *140*

Diversity among UK *Campylobacter jejuni N*CTC 11168 laboratory strains
WILLIAMS Lisa K., United Kingdom *141*

Genetic diversity of *Campylobacter* in wild birds
MAROTTA Francesca, Italy *142*

Comparative Genomics of selected oral and intestinal *Campylobacter concisus* strains
MOHSINA Huq, Australia *143*

Epigenome-based adaptive evolution: evidence from genomes, methylomes, transcriptomes and phenotype in *H. pylori*
KOBAYASHI Ichiz, France *144*

Pan-genomic analyses identify key *Helicobacter pylori* pathogenic loci modified by carcinogenic host microenvironments
NOTO Jennifer M., USA *145*

A large-scale comparative genomic survey of the *Campylobacter jejuni* population structure:towards the development of a nomenclature for global surveillance
DILLON Barker, Canada *146*

Integrated whole genome MLST and SNP analyses applied to publicly available *Campylobacter jejuni/coli* isolates
DE CONINCK Dieter, Belgium *147*

A stable and highly diverse genetic structure in clinical *Campylobacter jejuni* isolates in 2009 and 2015
ROSE Valérie, France 148

Campylobacter adaptation during acute and persistent human infections
CROFTS Alexander A., USA.....149

Impact of immunisation and bottlenecks on phase-variable gene expression patterns during colonisation of chickens by *Campylobacter jejuni* strain
LANGO-SCHOLEY Lea, United Kingdom 150

Could FlhF be a key element that controls *Campylobacter jejuni* flagella biosynthesis in the initial assembly stage?
FANGZHE Ren, China 151

Genomic analysis of the recently emerged antimicrobial resistant *Campylobacter jejuni* ST-6964 in New Zealand
ZHANG Ji, New Zealand 152

Genome-wide association of disease severity traits in *Campylobacter* from malnourished children in the Peruvian Amazon
BEN Pascoe, United Kingdom 153

Homologous recombination between genetically divergent reptile-associated *Campylobacter fetus* lineages
MAARTEN J. Gilbert, the Netherlands 154

Base-excising restriction enzymes towards epigenetic immune systems
ZHANG Y, Japan 155

Session « Pathogenicity and virulence factors »

Further investigation of the roles of fibronectin-binding proteins CadF and FlpA during *Campylobacter jejuni* interactions with intestinal epithelial cells
MAHJANAH Hussein, United Kingdom 157

The *Campylobacter jejuni* protein Cj0588 as a model system for investigating the role of TlyA methyltransferases in bacterial cell
SAŁAMASZYŃSKA-GUZ Agnieszka, Poland 158

Interactions between Dsb (disulfide bond) proteins of *Campylobacter jejuni* 81116
BOCIAN-OSTRZYCKA Katarzyna Marta, Poland.....159

Helicobacter pylori Secreted Protein HP1286 Triggers Apoptosis in Macrophages via TNF-Independent and ERK MAPK-Dependent Pathways
TAVARES Raquel, Sweden *160*

The bile salt sodium taurocholate induces *Campylobacter jejuni* outer membrane vesicle production and increases OMV-associated proteolytic activity
ABDI Elmi, United Kingdom *161*

Assembly of the Cytochrome c Oxidase in *Campylobacter jejuni*
GARG Nitanshu, United Kingdom *162*

Identification of *Helicobacter pylori* HP0231 (DsbK) targets by site-directed mutagenesis
BOCIAN-OSTRZYCKA Katarzyna Marta, Poland *163*

C8J1298 – *Campylobacter jejuni* dimeric oxidoreductase – oxidase or isomerase?
BANAŚ Anna M., Poland *164*

Impact of the selected amino acids, N- and C-terminal parts of HP0377 on its functioning as CcmG (cytochrome c maturation) protein
GRZESZCZUK Magdalena Joanna, Poland *165*

Investigation of the role of *Campylobacter jejuni* Type VI secretion system in secretion of effectors and interactions with host cells
JANIE Liaw, United Kingdom *166*

Campylobacter jejuni infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses
HAAG Lea-Maxie, Germany *167*

Blood adaptation mechanisms of *Campylobacter jejuni* associated with systemic infection
ZUOWEI Wu, USA *168*

C. *coli* clade 3 strains induce cell death in human HT-29 colon cells
JOHANSSON Cecilia, Sweden *169*

Campylobacter virulence in pathophysiology of post infection Irritable Bowel Syndrome
MADHUSUDAN Grover, USA *170*

Phase-variable epigenetic gene regulation modulates *Helicobacter pylori* motility and virulence factor expression
GORRELL Rebecca, Australia.....171

cj0371: a novel virulence-associated gene of *Campylobacter jejuni*
XUEQING Du, China 172

Helicobacter suis induces changes in gastric inflammation and acid secretion markers in pigs of different ages
DE WITTE Chloë, Belgium 173

Session «Immunology and Host response »

The intracellular proteins of *Helicobacter pylori* trigger immunomodulatory response in gastric epithelial AGS cells
CHAICHANAWONGSAROJ Nuntaree, Thailand.....175

Investigation of the Influence of Phase Variation on the Biological Phenotypes, Immunity to and Structure of the Flagella in *Campylobacter jejuni*
KHALOUD Alarjani, United Kingdom 176

Vaccination with a capsule conjugate or infection with *Campylobacter jejuni* induces serum bactericidal antibodies measured using a flow cytometric-based assay
SCHUMACK Nina M., USA.....177

Kaempferia parviflora inhibits inflammatory response mediated by *Helicobacter pylori* in human gastric cells
NEMIDKANAM Variya, Thailand.....178

Genomic origin for the variability of host-immune response triggered by *Helicobacter pylori*
BERTHENET Elvire, United Kingdom 179

Evidence for extra-intestinal spread of *Campylobacter* spp.; analysis of bird matched samples
WILLIAMS Lisa K., United Kingdom.....180

Diverse Responses of Human and Chicken Intestinal Epithelial Cells to a bacterial population within the species *Campylobacter jejuni*
DANIEL A. John, USA 181

Campylobacter colonisation in chickens: extra-intestinal spread linked to stress?
WILLIAMS Lisa K., United Kingdom.....182

Session « Antibiotics and antimicrobial resistance »

What affects antimicrobial resistance in *Campylobacter* along the broiler chicken supply chain?
DRAMÉ Ousmane, Canada 184

Antimicrobial resistance and characterization of gyrA mutation of *Campylobacter jejuni* and *Campylobacter coli* through commercial broiler production chains in Thailand
THOMRONGSUWANNANKIJ Thotsapol, Thailand 185

Comparative analysis of virulence and antimicrobial resistance of genetically different *Campylobacter* from poultry farms
GARCÍA-SÁNCHEZ Lourdes, Spain 186

Multilocus sequence typing of multiresistant *Campylobacter coli* strains isolated from turkey in Poland
KANIA Iwona, Poland.....187

Diversity of tet(O)-like determinants in *Campylobacter* spp. isolated from pigs in Scotland
LOPES Bruno, United Kingdom 188

Campylobacter jejuni isolated from pigs and cattle in Poland: genetic diversity and antibiotic resistance profiles
WIECZOREK Kinga, Poland.....189

Interconnection of antibiotic resistance, genetic proximity and the origin of *Campylobacter jejuni* isolates
SHAGIEVA Ekaterina, Czechia.....190

Molecular characterisation of tetracycline and ciprofloxacin resistant *Campylobacter jejuni* strains isolated from humans, poultry and wild birds
AKSOMAITIENĖ Jurgita, Lithuania 191

The heterogeneous response of isogenic *C. jejuni* NCTC11168 to erythromycin
OVSEPIAN Armen, Denmark.....192

A case of recurrent multidrug resistant *Campylobacter jejuni* bacteraemia in an immunocompromised host– clinical presentation and management
GODBOLE Gauri, United Kingdom *193*

Campylobacter jejuni and *Campylobacter coli* prevalence and antimicrobial susceptibility pattern in travelers' diarrhea in Thailand and Nepal
BODHIDATTA Ladaporn, Thailand *194*

Typing and resistance characteristics of *Campylobacter jejuni* and *Campylobacter coli* isolates from patients in Germany
FRUTH Angelika, Germany *195*

A pediatric case of enteritis associated with a *Campylobacter jejuni* strain resistant to gentamicin: Study of the resistance associated genes
PÉREZ-CATALUÑA Alba, Spain *196*

Acquisition of second mutation in gyrA caused high resistance to sitafloxacin in *Helicobacter pylori* after unsuccessful sitafloxacin containing treatment
MORI Hideki, Japan *197*

Diversity of *Helicobacter pylori* clarithromycin resistance in gastric mucus samples
LU Sun, China *198*

Mechanism of resistance to β-Lactams in *Helicobacter cinaedi*
RIMBARA Emiko, Japan *199*

Session « Emerging and Related Organisms species »

Rewriting the rulebook: minimal standards for describing new *Campylobacter* , *Arcobacter*, *Helicobacter* and *Wolinella* species
ON Stephen Lincoln, New Zealand *201*

The role of emerging Epsilonproteobacteria in childhood gastroenteritis
CORNELIUS Angela, New Zealand *202*

Could gastric microbiota be the cause of reactive gastropathy?
OKADA Takayuki, Australia *203*

First description of *Campylobacter lanienae* from feces of organic and conventional pigs, in France
KEROUANTON Annaëlle, France *204*

Investigation of the Function of T4SS and T6SS in *Campylobacter rectus*
BLACKBURN Dana, USA *205*

Updating the genomic taxonomy and epidemiology of *Campylobacter hyointestinalis*
WILKINSON David, United Kingdom *206*

Oxygen availability influences motility and biofilm formation of the emerging gastrointestinal pathogen, *Campylobacter concisus*
OVESEN Sandra, USA *207*

Investigating the virulence characteristics of the emerging pathogen *Campylobacter concisus*
ELTAHER Elshagmani, Australia *208*

Multi locus sequence typing and detection of virulence genes in *Campylobacter concisus* isolates from Denmark, using whole genome sequence data
KIRK Karina Frahm, United Kingdom *209*

Prevalence of *Campylobacter concisus* and *C. ureolyticus* in travelers' diarrhea cases and asymptomatic controls
SERICHANTALERGS Oralak, Thailand *210*

Frequency of *Campylobacter ureolyticus* in stools of patients with gastrointestinal disorders in a central Bohemian regional hospital
MAREJKOVÁ Monika, Czech Republic *211*

Past, Present and Future of the Taxonomy of the Genus *Arcobacter*
FIGUERAS MARIA José, Spain *212*

Analysis of 28 *Arcobacter* genomes belonging to different species
PÉREZ-CATALUÑA Alba, Spain *213*

Intestinal expression of genes encoding inflammatory mediators and gelatinases during *Arcobacter butzleri* infection of secondary IL-10 deficient mice
HEIMESAAT Markus M, Germany *214*

Arcobacter butzleri induce colonic, extra-intestinal and systemic inflammatory responses in secondary abiotic IL-10 deficient mice
GÖLZ Greta, Germany *215*

Survey of small intestinal and systemic immune responses following murine *Arcobacter butzleri* infection
HEIMESAAT Markus M, Germany.....216

Toll-like-Receptor-4 is essential for *Arcobacter butzleri* induced colonic and systemic immune responses in secondary abiotic IL-10-/- mice
GÖLZ Greta, Germany.....217

Toll-like Receptor-4 dependent small intestinal immune responses following murine *Arcobacter butzleri* infection
GÖLZ Greta, Germany.....218

Toll-like Receptor-4 dependent intestinal gene expression during *Arcobacter butzleri* infection of secondary abiotic IL-10 deficient mice
GÖLZ Greta, Germany.....219

Fitness cost of ciprofloxacin resistance in *Arcobacter butzleri*
FERREIRA Susana, Portugal 220

Detection of *Arcobacter* spp. along the intestinal tract of broiler chickens
SCHÖNKNECHT Antje, Germany 221

Comparison between the incidence of *Arcobacter* from water and shellfish using the MPN method and two culture methods in parallel
FIGUERAS MARÍA José, Spain 222

The Prevalence and Molecular Typing and Antimicrobial Resistance of *Arcobacter* spp. Isolated from Raw Chicken Meat in Beijing
ZHANG Mao-jun, China.....223

Prevalence of *Arcobacter* spp. in retail seafood in Germany
ZHANG Xiaochen, Germany.....224

Prevalence, genetic diversity and resistance to antibiotics of *Arcobacter* spp. from retail food from Portugal
VICENTE-MARTINS Sofia, Portugal 225

Isolation of *Okadaella gastrococcus* II sp. Nov. from a *Helicobacter pylori* positive Japanese patient with dyspepsia
OKADA Takayuki, Australia.....226

Gram stain reactions of *Okadaella gastrococcus* (Og): Gram stain negative to variable microorganism
OKADA Takayuki, Australia.....227

Whole-genome sequencing identifies three novel *Helicobacter* species isolated from gastric mucosa of red foxes
GRUNTAR Igor, Slovenia *228*

Non-pylori Helicobacters (NHPHs) induce shifts on gastric microbiota in *Helicobacter pylori*-infected patients
PENG Xianhui, China *229*

Session « Epidemiology and Public Health »

Use of genotyping tools to characterise and investigate the New Zealand Havelock North campylobacteriosis outbreak
CORNELIUS Angela, New Zealand *231*

Application of Whole Genome Sequencing for real time investigation of a *Campylobacter* outbreak associated with consumption of raw milk
SWIFT Craig, United Kingdom *232*

Consecutive waves of human *Campylobacter jejuni* infection associated with poultry identified by integrated whole genome surveillance in Luxembourg
RAGIMBEAU Catherine, Luxembourg *233*

Whole genome sequencing and empirical epidemiology identifies disparate etiologies between *Campylobacter jejuni* sequence types ST50 and ST61
STRACHAN Norval, United Kingdom *234*

Molecular Characterisation of *Campylobacter* in Humans and Chicken from Nigeria
AUDU Benshak J, United Kingdom *235*

Molecular characterization and antimicrobial resistance of *Campylobacter coli* strains isolated from different sources in Brazil
NOGUEIRA GOMES Carolina, Brazil *236*

Evaluating the epidemiological concordance of cgMLST clusters using the EpiQuant framework
HETMAN B., Canada *237*

Genetic Diversity of *Campylobacter upsalienisis* Strains Isolated in the US between 1989 and 2016
PRUCKLER Jan, USA *238*

A study elucidating the socio-demographics of *Campylobacter* infection in Scotland
ROTARIU Ovidiu, United Kingdom *239*

Domestic campylobacteriosis in Scandinavia from 2000 to 2015: trends in time and space
KUHN Katrin Gaardbo, Denmark *240*

What drives seasonality of human campylobacteriosis: dynamics of source contamination or of exposure to sources?
DAVID Julie, France *241*

Finding outbreaks in the ever-changing notification haystack: campylobacteriosis surveillance in two regions in New Zealand
MARSHALL Jonathan, New Zealand *242*

Human campylobacteriosis resulting from continuous source outbreaks in the United Kingdom
CODY Alison, United Kingdom *243*

Exploring *Campylobacter* surveillance data with visual analytics
VARGA Margaret, United Kingdom *244*

Chicken persists as the dominant source of *Campylobacter* in the United Kingdom (UK)
JANSEN VAN RENSBURG Melissa J., United Kingdom *245*

Chickens in the city, cows and sheep in the country: A simple model for the source of campylobacteriosis
SIH JING Liao, New Zealand *246*

Calves as source of infection with *Campylobacter* in a farm worker
HANSSON Ingrid, Sweden *247*

Insight into Consumer Phase Practices Regarding Chicken Meat Consumption, Handling, and Preparation in Egypt
IHAB Habib, Australia *248*

Modelling *Campylobacter* infection dynamics in young children in low-resource settings
NITYA Singh, USA *249*

Colon cancer risk in patients with a history of *Campylobacter* infection
Lapo Mughini-Gras, the Netherlands *250*

Increased risk of inflammatory bowel disease after *Campylobacter concisus* and *Campylobacter jejuni* infection; a Danish population-based cohort study
HANS Linde Nielsen, Denmark *251*

Campylobacter pancolitis Mimicking Ulcerative colitis
TEA HOON Kim, South Korea *252*

CHRO are highly relevant with the Global Water Pathogen Project
MILLER Jennifer, USA *253*

Session « Methods for Detection, Identification and Characterisation »

Development of "Recommendations for the Diagnosis of *Campylobacter* Infection by Clinical and Public Health Laboratories"
AUBERT Rachael D., USA *255*

Using Pan-genomes for Molecular Assay Design: Development of a Multiplex Ligation-dependent Probe Amplification (MLPA) Assay for the Detection of 28 Epsilonproteobacterial Taxa
CORNELIUS Angela, New Zealand *256*

Characterization of the heme uptake regulator, HeuR, in *Campylobacter jejuni*
BRITTNI Kelley, USA *257*

Comparison of PFGE, MLST and MALDI-TOF, for typing *Campylobacter coli* and relation with the virulence of these strains
DENIS Martine, France *258*

Evaluation of a core genome multilocus sequence typing scheme for *Campylobacter* outbreak investigation
KÖBERL-JELOVCAN Sandra, Austria *259*

Whole Genome Sequencing of *Campylobacter* for Routine Surveillance
PAINSET Anaïs, United Kingdom *260*

Evaluation of Whole Genome Sequencing for *Campylobacter jejuni* Surveillance and Outbreak Detection
JOSEPH Lavin, USA *261*

Development and Standardization of a Whole Genome Multi-locus Sequence Type (wgMLST) Database for *Campylobacter* Characterization
WILLIAMS Grant, Belgium *262*

Something old, something new: added value of a vintage culture technique for emerging enteric pathogen detection.
VAN DEN ABEELE Anne-Marie, Belgium *263*

Orion GenRead *Campylobacter*. A new kit for a rapid detection of campylobacters in stools.
MEGRAUD Francis, France *264*

Surveillance of *Campylobacter ureolyticus* in Japan and development of multiplex PCR to detect C. *jejuni*, C. *coli* and C. *ureolyticus*
SHIMIZU Akinori, Japan *265*

Recombinase Polymerase Amplification (RPA) for the detection of *Campylobacter* in chicken faeces
ACHTERBERG René, The Netherlands *266*

Detection of *Campylobacter fetus* in faeces samples from small ruminants using real-time PCR and MALDI-TOF
GREETJE A.A. Castelijn, the Netherlands *267*

Development of internal sample process control for quantification of live *Campylobacter* on broiler meat by real-time PCR
PACHOLEWICZ Ewa, Germany *268*

Evaluation of *Campylobacter* isolation methods for samples collected from poultry farms and slaughterhouses in Thailand
PHETSRI Natnicha, Thailand *269*

Selective Medium for Growth of *Campylobacter* in Containers Incubated Aerobically
HINTON Arthur, Jr., USA *270*

Evaluation of C. *jejuni* strains and mCCDA batches for testing the performance of the media
NAGARD Bérengère, France *271*

Comparison of four different selective media for the quantification of *Campylobacter* in poultry meat and rapid confirmation of suspect colonies
DE ZUTTER Lieven, Belgium *272*

Evaluation of a new method for the enumeration of *Campylobacter* in chicken carcass rinse samples.
DESCHOMETS Remy, France *273*

Publication of revised International Standard ISO 10272 for detection and enumeration of *Campylobacter* in the food chain
JACOBS-REITSMA Wilma, The Netherlands..... *274*

Development of a quantitative and qualitative chemotaxis assay, tHAP, a modified hard plug assay, using *Campylobacter jejuni* model
BASSAM Elgamoudi, Australia.....*275*

New colorimetric aptasensor for rapid on-site detection of *Campylobacter jejuni* and *Campylobacter coli* in chicken carcass samples
YOUNG-JI Kim, South Korea.....*276*

Violet-blue light as a novel probe to reveal mechanisms of oxidative damage in *Campylobacter jejuni*
WALKER Peter, United kingdom *277*

Development and Application of Oxidative Stress Sensing and Response Fluorescent Proteins in *Campylobacter jejuni*
DAI Lei, USA *278*

Can a visual loop-mediated isothermal amplification assay stand out in different detection methods when monitoring *Campylobacter jejuni* from diverse sources of samples?
XIAOQI Zang, China *279*

Comparison of liquid growth medias for cultivation of *Campylobacter concisus*
AAGAARD Marta Emilie, Denmark *280*

Rapid and specific methods to detect *Campylobacter hepaticus,* causing spotty liver disease in chickens, and differentiate from other human *Campylobacter*
VAN T.T.Hao, Austria *281*

Gastric juice-based real-time PCR for tailored *Helicobacter pylori* treatment: A practical approach
PENG Xianhui, China.....*282*

Detection of *H. pylori* in gastric biopsy material
WEND-HANSEN Clare, United Kingdom *283*

Session « Adaptation of *Campylobacter* spp. to environmental conditions »

Adaptive response to environmental conditions: the case of an atypical aero-tolerant *Campylobacter jejuni* strain
RODRIGUES Ramila C., France *285*

Identification of genes associated with environmental resistance in *Campylobacter jejuni* isolates from processing in a broiler slaughterhouse
CARBONERO A., Spain *286*

To give and take: *Campylobacter* spp. and the microbiome
HILBERT Friederike, Austria *287*

Persistence of *Campylobacter jejuni* in raw milk– insights from whole-genome sequencing
JAAKKONEN Anniina, Finland *288*

Dynamics, architecture and matrix of biofilms are different according to strains of *Campylobacter jejuni*
TURONOVA Hana, Czech Republic *289*

wgMLST and *in vitro* study of biofilm formation in two *Campylobacter jejuni* populations isolated from a poultry slaughterhouse environment
GARCÍA-SÁNCHEZ Lourdes, Spain *290*

Survival variability of *Campylobacter jejuni* strains subjected to stressful conditions
DUQUÉ Benjamin, France *291*

Thermotolerant *Campylobacter* spp: detection and enumeration during anaerobic digestion of livestock effluents in 5 biogas plants
REPÉRANT Elisabeth, France *292*

The ovine gallbladder: a protected niche for *Campylobacter jejuni*?
KREUDER Amanda J., USA *293*

Copper management and transportation in *Campylobacter jejuni*
GARG Nitanshu, United Kingdom *294*

Session « Poultry and non-poultry epidemiology and ecology of *Campylobacter* spp. »

The population structure of *Campylobacter* colonising broiler breeder flocks.
COLLES Frances M., United Kingdom *296*

Prevalence and genetic characterization of *Campylobacter* spp. in poultry farms in the North of Spain during two season periods
GARCÍA-SÁNCHEZ Lourdes, Spain *297*

Occurrence and genotypes of *Campylobacter* species in broilers during the rearing period
XIAOYAN Zhang, China *298*

Characterization of *C. jejuni* and *C. coli* broiler isolates by whole genome sequencing
CANTERO Guillermo, Spain *299*

On-farm risk factors associated with *Campylobacter* prevalence in conventional broiler flocks in the United States
SAHIN Orhan, USA *300*

The Fly hypothesis for *Campylobacter* transmission revisited
NICHOLS Gordon, United Kingdom *301*

The *Campylobacter jejuni* contamination level in houseflies after exposure to contaminated faeces
JENSEN Annette Nygaard, Denmark *302*

Glucose utilisation by *Campylobacter jejuni* strains isolated from farm associated Norway rats
MOHAMMED Othman, United Kingdom *303*

Multilocus sequence typing of *Campylobacter jejuni* along poultry food chain in Poland
OSEK Jacek, Poland *304*

Quantitative analysis of *Campylobacter* spp. contamination in chicken slaughtering lines by “label tracking method” in eastern China
JINLIN Huang, China *305*

Investigations into the association of *Campylobacter* contamination in chicken carcase and caeca samples in the United Kingdom
JOHN Rodgers, United Kingdom *306*

Identification of genetic determinants that facilitate *Campylobacter jejuni* survival during poultry processing and storage
SHANK Janette M., USA *307*

Quantitative surveys of *Salmonella* and *Campylobacter* on retail raw chicken in Yangzhou, China
JINLIN Huang, China *308*

Campylobacter spp. in retail poultry and bovine meat in Italy
DI GIANNATALE Elisabetta, Italy *309*

Campylobacter spp. prevalence and levels of contamination in poultry meat and bovine meat preparations marketed in Italy
SANTARELLI Gino Angelo, Italy *310*

Quantitative Relative Risk Characterization of *Campylobacter* Contamination in Chicken Meat from Formal and Informal Urban Markets in Alexandria, Egypt
IHAB Habib, Australia *311*

Host origin predicts colonization ability in *Campylobacter jejuni*
MOURKAS Evangelos, Sweden *312*

Occurrence of zoonotic *Campylobacter* species in cattle and sheep farms
OCEJO Medelin, Spain *313*

Campylobacter jejuni at the level of a coastal catchment in France
GOURMELON Michèle, France *314*

Prevalence and antimicrobial resistance of *Campylobacter* spp. found in gulls, Laridae, wintering in Zagreb, Croatia
JURINOVIĆ Luka, Croatia *315*

Genetic diversity of *Campylobacter coli* and *Campylobacter lanienae* from wild boars from a metropolitan area in northeastern Spain
CASTILLO-CONTRERAS Raquel, Spain *316*

Antimicrobial susceptibility and putative virulence factors among campylobacteria isolated from animals in Valparaiso, Chile
LEVICAN Arturo, Chile *317*

Characterization of *C. jejuni* isolates from western jackdaws
KOVANEN Sara, Finland *318*

Session « Control strategies for *Campylobacter* spp. »

Campylobacter colonization in broilers raised under different management concepts
KOENE Miriam, The Netherlands.....320

Efficient prevention of *Campylobacter* spp. entrance in broiler houses improves flock performance at slaughter
MCKENNA Aaron, United Kingdom 321

Optical flow predicts *Campylobacter* colonisation of broiler flocks at 7-10 days of age.
COLLES Frances M., United Kingdom.....322

Vaccination with nanocapsules derived from the ureA protein can induce protective immune responses in mice.
AYA Taki, Australia 323

Development of enterobactin antibody-based immune intervention strategies
XIMIN Zeng, USA.....324

A proof of concept study demonstrates the reduction of *Campylobacter* levels using bacteriophages
WIYADA Estella, Australia.....325

Application of Bacteriophage to Control Contamination of *Campylobacter* in Chicken Liver
FIRLIEYANTI A.S., United Kingdom.....326

Bacteriophage therapy to reduce *Campylobacter jejuni* colonization in broiler chickens
GIUSEPPE Aprea, Italy.....327

Identification of genetic determinants that facilitate *Campylobacter jejuni* survival during poultry processing and storage
SHANK Janette M., USA 328

Effect of a combination of two feed additives against *Campylobacter* infection in commercial turkey farms
QUESNE Ségolène, France 329

Combination of water and feed additives against *Campylobacter* in free-range broilers
GUYARD Nicodème Muriel, France.....330

Antimicrobial activity of a novel commercial antimicrobial (Auranta 3001), against T6SS positive *Campylobacter jejuni* and *Campylobacter coli*
ALEXANDROS Stratakos Ch., United Kingdom.....*331*

The mechanism of anti-*Campylobacter* activity of phytochemical formulations from alpine and karst plants, traditionally used against intestinal disorders
ŠIMUNOVIĆ Katarina, Slovenia.....*332*

The Effects of Thai-herb on *Campylobacter* colonization
JARRIYAWATTANACHAIKUL Watanya, Thailand.....*333*

Efficacy of formulated carvacrol on *Campylobacter jejuni in vitro* and electronic microscopy approaches
ALLAOUA Marion, France.....*334*

Campylobacter species contamination in UK fresh whole raw chickens and associated packaging at retail sale
ELVISS Nicola, United Kingdom.....*335*

Diamond Like Carbon Ag Nanocomposites as a control measure against *Campylobacter jejuni* and *Listeria monocytogenes* on food preparation surfaces
ZAKARIENĖ Gintarė, Lithuania.....*336*

Proficiency testing organised by the European Union Reference Laboratory (EURL) for *Campylobacter* – the work before, during and after
SVENSSON Linda, Sweden.....*337*

Assessment of microbiological criteria for *Campylobacter* in poultry carcasses
LANNETTI Simona, Italy.....*337*

Plenary session
« Genomic and Evolution 1 »

Chairpersons:
SHEPPARD Sam, United Kingdom and DE REUSE Hilde, France

Genome-wide identification of Cancer associated genetic elements in *Helicobacter pylori*

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Introduction: *Helicobacter pylori* is present in about 50% of the global population. This coloniser of the human stomach is associated with gastric diseases such as gastritis and gastric ulcers. It is also the main cause for gastric cancer, the world's third most common cause of death by cancer. Large numbers of people carry this organism asymptomatically and many questions remain about why serious symptoms develop in a subset of infected humans. *H. pylori* has a high recombination rate, and this efficient source of gene acquisition might be central to both its colonisation success and the variability of outcomes in infected humans.

Methods: A dataset of 565 *H. pylori* genomes, from strains collected world-wide, was analysed using FineStructure to quantify population structure. The largest sub-population from the FineStructure analysis, with available patient data, was used in a genome-wide association study (GWAS). Genetic elements that were over-represented in strains from cancer patients compared to strains from non-cancer patients were identified with comparison to the clonal frame of the bacterial isolates, as well as ones over-represented in strains from non-cancer patients.

Results: This analysis identified SNPs and accessory variation in a number of *H. pylori* genes associated with cancer or benign inflammation in the human host including known virulence factors such as *CagPAI* and *babA* genes.

Conclusion: Co-habitation between humans and *H. pylori* has taken place for millennia but recent antibiotic treatment regimens have resulted in increased resistance issues, while disturbing this co-evolution balance. Our results demonstrate that bacterial factors have a sufficiently strong influence on disease progression that even a small scale GWAS can identify them. Therefore, *H. pylori* GWAS can elucidate mechanistic pathways to disease and guide clinical treatment options, including for asymptomatic carriers.

Genomic and population structure analysis of *Helicobacter pylori* in China reflect geography specific lineages corresponding to historic East Asian migrations

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Background: In China, the average infection rate of *Helicobacter pylori (H.pylori)* is about 59%. Despite the high prevalence of *H.pylori*, limited information on the genomic characterization related to migrations of this pathogen have been reported in China.

Materials and Methods: As the largest center for *H.pylori* strain collection and storage, here we sequenced 59 strains isolated from gastritis, digestive ulcer and gastric carcinoma patients and from various geography and ethnic group in China. In combination with six public available genomes, we constitute a dataset containing 65 *H.pylori* genomes of China. Comparative genomic analysis are performed to get core and pan genomes, as well as the strain specific genes. The *H.pylori* China dataset is further extended to include genomes from other east Asian countries such as Korea, Japan, Malaysia. Phylogenetic tree is constructed based on the core genome SNPs. Moreover, genetic flux and population structure are estimated based on a co-ancestry matrix generated from Chromopainter followed by fineSTRUCTURE analysis.

Results: The number of core gene is 1147, pan genes are 3072, strain specific genes ranged from 0 to 47.A phylogenetic tree analysis based on 237,250 core genome SNPs of East Asian dataset divides Chinese *H. pylori* populations into three lineages according to different geographies. We generated a co-ancestry matrix for hspEastAsia containing 16 subpopulations with more than one strain, among which a complex genetic flux was found. Some subpopulations could be preliminarily inferred to correlate with several ancient migrations of Chinese population: going to the South Sea, rush to the northeast of China and the migrations by Tea-horse ancient road. These movements are also part of the activities in the Maritime silk road and Land silk road.

Conclusions: The fine population structure based on this genomic data provides significant and attractive insights into the population genetics of hspEastAsian subgroup and corresponding historic migration events.

Putting bacterial GWAS to the test:
can we find the basis of host-association in *Campylobacter jejuni*?

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2016 was a significant year for bacterial genome-wide association studies (GWAS). It saw the release of two new *k*-mer-based methods and was said to herald the coming of age of microbial GWAS. However, with few but notable exceptions, bacterial GWAS has only been applied to detect the genetic underpinnings of antibiotic resistance and there are plenty of reasons to look on these as "easy" traits: Antibiotic resistance is typically under strong fluctuating selection, rapidly increasing and decreasing with the presence or absence of the antibiotic, emerging and disappearing from multiple independent backgrounds. In other words, they are ideal traits to study with GWAS. However, the truly revolutionary promise of GWAS is the ability to elucidate the genetic basis of far more complex traits. Putting bacterial GWAS to the test, we posed a challenging question: What is the genetic basis for host-association in *Campylobacter jejuni*?
To answer this question, we whole-genome sequenced 408 *C. jejuni* strains isolated from chickens and 301 strains isolated from a diverse set of wild birds and applied a phylogeny-based GWAS method previously used to detect the genetic basis of biofilm formation and the genetic elements that increases in frequency during poultry processing. We found several genes and gene variants associated with chickens, including genes encoding the three subunits of the cytolethal distending toxin (CDT), a methyl-accepting chemotaxis protein (MCP) and several genes associated with the flagellar hook. These results demonstrate how bacterial GWAS can be applied to generate testable hypotheses even for complex traits such as host-association.

Whole genome sequencing of *Campylobacter jejuni* offers an opportunity for in depth investigation into phase variable

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Phase variable genes play an important role in the expression as well as the structure of surface proteins. As such, they can influence host adaptation and virulence. In *Campylobacter jejuni*, the presence of variable poly G/C tracts can result in phase variation. Several genes have been reported to contain such tracts, but up to now, only a limited number of strains have been included in this type of studies.

The increased availability of whole genome sequences creates an opportunity for a more extensive investigation of phase variable genes in *Campylobacter jejuni*.

A scheme containing 3529 different genes previously found in *Campylobacter jejuni/coli* were used to screen a set of more than 1000 genomes using the wgMLST functionality of BioNumerics 7.6. The database containing all alleles per locus was then screened to identify loci with poly G/C tracts of different lengths.

Several genes with variable poly G/C tracts were identified. This included genes identified in previous studies, as well as several additional genes. Poly G tracts towards the end of the gene were most common. Variation in the length of these tracts was even found in repeated passages of the same isolate, further implicating this mechanism in host adaptation and virulence.

Morphological transformation of *Helicobacter pylori* upon expression of a small peptide by a type I toxin-antitoxin system

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Toxin antitoxin systems (TAS) have been identified in a wide range of bacterial chromosomes and plasmids with various roles ranging from plasmid stabilization to biofilm formation or bacterial persistence. In type I TAS, the synthesis of a toxin is counteracted by base pairing of the toxin mRNA with its cognate antitoxin RNA leading to the degradation of the RNA duplex.
Here, we report the characterization of one of the six class A type I TAS (AapA1-isoA1) expressed from the chromosome of the major human gastric pathogen, *Helicobacter pylori*. The AapA1 locus encodes the toxin, a 30 amino acid peptide whose expression leads to a rapid growth arrest associated with a total change of *H. pylori*'s morphology from actively growing spiral-shaped cells to coccoid bacteria.
H. pylori coccoids are viable but non-culturable forms that have been observed both in gastric biopsies of patients, during *in vitro* growth in late stationary phase or in response to diverse stresses. The biological function of these forms is still under debate.
Using fluorescence live microscopy, we monitored at the single cell level the coccoid conversion upon toxin over-expression. Our data indicate that the toxin-induced conversion is not a time-dependent process but is rather directly associated to interference with cell division. These results were consistent with CryoEM observations of the coccoids. Moreover, we showed that the AapA1 toxin targets specifically the bacterial inner membrane without causing visible disruption, but instead moderately lowers the intracellular ATP concentration and locally influences the membrane potential. Using AapA1 toxin and isoA1 antitoxin promoter reporter fusions, we observed that oxidative stress represses antitoxin expression leading to an imbalanced ratio in favor of the AapA1 toxin expression.
Finally, a *H. pylori* mutant strain carrying a deletion of each of the six class A TAS was constructed. Using this mutant, we are now investigating whether these TAS are involved in bacterial persistence.

Genome and methylome variability of cag pathogenicity island-carrying *Helicobacter pylori* during early human infection

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Helicobacter pylori is characterized by remarkable genetic diversity. Yet, little is known about the evolution of the *H. pylori* genome during the initial phase of infection. In the present study, we investigated genome and methylome variation of *H. pylori* during an experimental human infection with a fully virulent *H. pylori* strain. Twelve volunteers were treated with a prophylactic vaccine candidate containing CagA, VacA, and NAP, or placebo, respectively, and subsequently challenged with the *cag*PAI-positive *H. pylori* strain BCM-300. The genomes of the challenge strain and 12 reisolates obtained 12 weeks after infection were sequenced by SMRT® sequencing technology. Whole genome comparisons revealed an average mutation rate of 5.2×10^{-6} mutations per site per year. A loss of *cag*PAI functionality was observed in three of the reisolates. In addition, three reisolates of the vaccine group acquired mutations in the vacuolating cytotoxin gene *vacA* resulting in a premature stop codon. The specific inactivation of *vacA* in the vaccine group points to a functional involvement of this loss of VacA in evasion of the vaccine-induced immune response. The methylome analysis identified 15 methylated motifs. Eleven of these were assigned to known methyltransferase (MTase) activities. Insertion mutagenesis enabled the assignment of further three of these sites to functionally uncharacterized MTase genes. Inter-strain variability in the methylomes was observed, which was found to result from phase variation of MTase genes. The epigenetic changes via phase variation of MTase genes might confer a selective advantage in adaptation to different human hosts. Our study of adaptation of a fully virulent *H. pylori* strain to 12 different human hosts permits the most robust estimate yet of the frequency of genetic changes in the absence of inter-strain recombination, and highlights the relevance of the extraordinary genetic variability of *H. pylori* as a challenge in vaccine development.

Parallel session
« Immunology and Host response 1 »

Chairpersons:
ETHELBERG Steen, Denmark and FERRERO Richard, Australia

Regulation of the LATS2/YAP/TAZ pathway during *Helicobacter pylori* induced gastric carcinogenesis

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Introduction: Cancer stem cells (CSC) have been identified in gastric carcinoma, in which they control tumor initiation, growth and dissemination. We reported that *Helicobacter pylori* infection leads through an epithelial-mesenchymal transition to the emergence of CD44+ cells with CSC properties. The YAP/TAZ co-transcription factors of the Hippo pathway control cancer initiation and progression in many cancers, but their regulation in the context of *H. pylori* mediated gastric carcinogenesis has not been described.

Materials and Methods: This work aimed to study the role of the Hippo/YAP/TAZ pathway during *H. pylori* mediated EMT and emergence of CSC-like cells. Coculture experiments of MKN74, MKN45 and AGS gastric cell lines with the 7.13 *cag*A+ and *cag*A mutant *H. pylori* strains were performed. We evaluated YAP/TEAD transcriptional activity and YAP/TAZ/TEAD target genes expression (RT-qPCR). The consequences of LATS2 and YAP/TAZ inhibition by siRNA and by a YAP/TEAD inhibitor were evaluated on *H. pylori*-induced EMT and CSC properties (tumorspheres). The expression of Hippo/YAP/TAZ components we evaluated in gastric tissues (immunohistochemistry) of patients infected or not with *H. pylori*, and with gastric carcinoma.

Results: *H. pylori* via CagA induced a rapid activation of YAP/TAZ and upregulation of their target genes, which remained under the tight control of LATS2. YAP/LATS2 overexpression was also detected in *H. pylori*-infected gastric mucosa. *In vitro,* YAP/TAZ inhibition reduced EMT/CSC markers expression, and efficiently reduced the pool of CD44+ gastric CSCs forming tumorspheres. In gastric carcinoma, YAP and LATS2 were co-overexpressed in 5-30% of cells, which may correspond to gastric CSCs.

Conclusion: YAP/TAZ and LATS2 are activated in *H. pylori*-infected gastric epithelial cells and their sustained activity controls EMT and CSC properties, constituting a promising target to inhibit gastric carcinogenesis.

Interleukin-18 mediates immune responses in *Campylobacter jejuni* infected secondary abiotic mice

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Background: Human *Campylobacter jejuni* infections are progressively rising worldwide. Informations about the molecular mechanisms underlying campylobacteriosis, however, are limited. In the present study we investigated whether cytokines such as IL-23, IL-22 and IL-18 sharing pivotal functions in host immunity were involved in mediating intestinal and systemic immunopathological responses upon *C. jejuni* infection.

Methodology/Results: To assure stable infection, secondary abiotic IL-23p19-/-, IL-22-/- and IL-18-/- mice were generated by broad-spectrum antibiotic treatment. Following peroral *C. jejuni* strain 81-176 infection, mice of either genotype harbored comparably high pathogenic loads in their intestines. As compared to wildtype controls, however, IL-18-/- mice displayed less distinct *C. jejuni* induced sequelae as indicated by less pronounced large intestinal shrinkage and lower numbers of apoptotic cells in the colonic epithelial layer at day 8 postinfection (p.i.), that were accompanied by lower colonic numbers of adaptive immune cells including regulatory T cells and B lymphocytes and less distinct secretion of pro-inflammatory cytokines such as TNF, IFN-gamma, and IL-17A in colonic *ex vivo* biopsies at day 8 p.i. Upon *C. jejuni* infection, colonic IL-23p19 expression was up-regulated in IL-18-/- mice only, whereas IL-22 mRNA levels were lower in naive and infected IL-23p19-/- as well as infected IL-18-/- as compared to respective wildtype control mice. Remarkably, not only intestinal, but also systemic infection-induced immune responses were less pronounced in IL-18-/- mice as indicated by lower TNF, IFN-gamma, and IL-6 serum levels as compared to wildtype mice.

Conclusion: We here show for the first time that IL-18 is essentially involved in mediating *C. jejuni* infection in the secondary abiotic mouse model. Future studies need to further unravel the underlying regulatory mechanisms orchestrating pathogenic-host interaction.

Campylobacter jejuni induces autoimmune peripheral neuropathy _via_ Siglec-1 and IL-4 axes in a mouse model of Guillain Barré Syndrome

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Campylobacter jejuni is a Gram-negative bacterium that is the most common bacterial cause of gastroenteritis worldwide. *C. jejuni* infection has also been causally linked with development of the peripheral neuropathy called Guillain Barré Syndrome (GBS). We have previously shown that *C. jejuni* isolates from human enteritis patients induce a Type1/17 cytokine dependent colitis response in IL-10$^{-/-}$ mice. In contrast, isolates from human GBS patients colonize the IL-10$^{-/-}$ mice without inducing colitis but instead induce autoantibodies targeted against peripheral nerve gangliosides. We hypothesized that *C. jejuni* from GBS patients induce autoimmune responses and nerve lesions dependent upon IL-4 and Siglec-1 axes. C57BL/6 IL-10$^{-/-}$ mice were gavaged orally with *C. jejuni* strain HB93-13 or 260.94 from GBS patients, half given blocking antibodies for IL-4 or Siglec-1 and all assessed for clinical neurological signs/phenotypes, anti-ganglioside antibodies, cellular immune responses and lesions in gut and peripheral nerve tissues. Vehicle inoculated mice served as infection controls, while mice given isotype control antibody served as controls for IL-4 and Siglec-1 blocking antibody treatments. *C. jejuni* HB93-13 and 260.94 infection elicited mild GBS in mice. Antiganglioside antibody responses were dependent upon blunted Type1/17 but enhanced Type2 cytokine production by T helper cells. Autoantibody production correlated with enhanced macrophage infiltration in sciatic nerves and their dorsal root ganglia. Autoantibodies and histological lesions were significantly decreased in mice depleted of IL-4, without leading to induction of colitis. Peripheral nerve lesions were mild in infected mice, but were associated with abnormal gait and hind limb movements consistent with this syndrome's manifestation in humans. Moreover, Siglec-1 served as a central antigen presenting cell receptor mediating GBS but not colitogenic isolate uptake, T cell differentiation and autoantibody elicitation. Thus, this is the first mouse model of an autoimmune disease induced directly by a bacterium that is dependent upon Siglec-1 and IL-4 axes.

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Downregulation of USF1 transcription factors impacts p53 during *Helicobacter pylori* infection and exacerbates gastric carcinogenesis

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Introduction: *Helicobacter pylori* is the major risk factor for gastric cancer. It induces genetic instabilities in gastric epithelial cells associated to chronic inflammation and impairs host DNA repair systems. Its oncoprotein CagA promotes the proteasomal degradation of p53, a central regulator of the DNA damage response. We previously reported that *H. pylori* inhibits the expression of the genes coding for the upstream stimulating factors USF1 and USF2 by DNA hypermethylation. USF belong to the basic helix-loop-helix-leucine zipper (bHLH-zip) class of transcription factors. USF1 regulates DNA damage response and the expression of *Tp53*. In addition, in response to a genotoxic stress USF1 binds to p53 and prevents its proteasomal degradation. In the present study, we investigate the consequences of the deregulation of USF1 on p53 level and DNA damage and repair response during *H. pylori* infection. We also address the impact of loss of USF1 on the severity of gastric cancer lesions induced by *H. pylori*.

Methods: Gastric epithelial cells (MKN45/AGS) were infected with *H. pylori* (7.13/26695) for 2h/24h and/or treated with camptothecin (CPT) a genotoxic compound. USF1 and p53 levels, their cellular localization and USF1-p53 interaction were analysed by immunofluorescence and Duolink proximity ligation assay. The consequences of USF1-deficiency were investigated on gastric lesions-induced by *H. pylori* in *Usf1*$^{-/-}$ and *Usf1*$^{+/+}$ mice after 9/12 months.

Results: *H. pylori* inhibits USF1 nuclear level and translocates USF1 in the cytoplasm. Concomitantly, p53 is depleted in the nuclei. *H. pylori* inhibits USF1-p53 interaction induced by CPT, and consequently favors p53 degradation. In addition, the severity of *H. pylori*–induced gastric lesions is exacerbated in Usf1$^{-/-}$ mice compared to Usf1$^{+/+}$.

Conclusions: USF1 is a central regulator of the DNA damage response to *H. pylori* infection. Its deficiency is directly associated with p53 degradation and the promotion of the gastric carcinogenesis process.

Parallel session
« Methods for Detection, Identification and Characterisation »

Chairpersons:
T*ABODOA Eduardo, Canada, RIVOAL Katell, France and BESSEDE Emilie, France*

A core genome multi-locus sequence typing scheme for stable, comparative analyses of *Campylobacter jejuni* and *C. coli* human disease isolates.

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Introduction: The epidemiology of human campylobacteriosis outbreaks has remained poorly defined largely due to the lack of high resolution isolate characterisation methods. However, the number of loci available for analysis has been substantially increased by the availability of whole genome sequence data. Our aim was to develop a core genome MLST (cgMLST) scheme that defines a comprehensive set of loci for high-resolution comparisons across groups of genetically diverse isolates.

Material and Methods: Contiguous sequences from draft genomes of 2,472 clinical *C. jejuni* (2,207) and *C. coli* (265) isolates were scanned by BIGSdb software and the positions of the 1,643 loci, defined by the re-annotation of NCTC 11168, were recorded ('tagged') in each. Loci found to be absent in no more than five percent of isolates were included in the scheme, and paralogous loci were identified and removed. The scheme was validated using draft genomes from 1,574 clinical and 1,371 non-clinical isolates deposited in the PubMLST.org/campylobacter database and by re-analysis of a known outbreak cluster.

Results: Of the 1,643 NCTC 11168 coding sequences, 1,365 were identified in ≥ 95% of 2,472 Oxfordshire clinical genomes; 22 of these were identified as paralogous loci and removed from the scheme. Ninety-five percent or more of the 1,343 cgMLST scheme loci were identified in 1,478 clinical genomes with ≤150 contigs, used for validation, of which 1,452 (98.2%) had alleles designated. For the 1,278 (93.2%) non-clinical genomes with ≤150 contigs, 1,222 (95.6%) had 95% or more cgMLST loci identified, of which 1,200 (93.9%) had designated alleles. Single-linkage cluster analysis of cgMLST profiles clearly distinguished 20 outbreak genomes from contemporaneous disease isolates.

Conclusion: This cgMLST gene set provides a high-resolution analysis scheme for human campylobacteriosis isolates which can be used for on-going disease surveillance, and the resolution of very closely related isolates obtained during outbreak investigation.

Comparison of nine selective agars for the detection of *Campylobacter* spp.

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The purpose of this study was to compare selective agars for the detection of *Campylobacter* spp. in poultry samples (meat or skin) using the NF EN ISO 10272-1 (2006) method. The media used for this comparison were mCCDA (the mandatory medium for this method), Karmali (KAR), Butzler n°2 (B2), Brilliance campycount (BCC), CASA, Campyfood (CFA), Campylosel (CS), RAPID'Campylobacter (RC) and CHROMagar Campylobacter (CAC). The samples were artificially contaminated with different strains (*C. jejuni, C. coli* and *C. lari*) at various concentrations.

The results were obtained from annual *Campylobacter* proficiency tests. The 1st comparison in 2015 included 17 French labs and mCCDA, CASA, CFA and RC were tested. The 2nd comparison in 2016 included 15 French labs and mCCDA, B2 and CAC were tested. The 3rd comparison was performed 3 times in our lab in 2017 on three distinct assays and mCCDA, B2, CAC, CASA, CFA, KAR, BCC and CS were tested.

No difference between the nine media was observed for detecting positive samples. They were all able to detect *Campylobacter* in more than 80% of the positive samples whatever the strain or concentration of the strain.

For negative samples, mCCDA was the weakest selective media with growth of annex flora on more than 50% of the plates. While CASA and Butzler n°2 were the most selective media; absence of growth of annex flora was observed on more than 90% of the plates for these two media.

Our results confirm the need to use a second selective media different from mCCDA to facilitate the detection of *Campylobacter*, as it will be asked for in the upcoming release of ISO 10272.

Evaluation of rapid immunochromatographic test detecting *Campylobacter* in human stools

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Diagnosis of Campylobacter infection is currently based essentially on stool culture. Several studies have shown that culture has a limited sensitivity. Today, new Campylobacter detection methods are available. Among them, immunochromatographic tests (ICTs) are very attractive because they give a result within 15 minutes. However, the specificity of the ICTs needs to be explored: in this study, the Ridaquick (r-Biopharm) and the ImmunoCardSTAT!Campy (Meridian) were evaluated in comparison to a composite reference test.

All stools of the emergency rooms patients were tested with the 2 ICTs as well as by culture. Samples detected as positive with one or the 2 ICTs and 153 stool specimens negative with the 2 ICTs were also tested with 2 ELISAs, Ridascreen (r-Biopharm) and PremierCampy (Meridian), and with an in-house real-time PCR.

A patient was considered to be Campylobacter positive if the culture was positive and, in the case of a negative culture, if an ELISA and the real-time PCR were positive simultaneously.

During the study period, 305 patients were included. The majority were children (65%) and males (57%). The main symptom was diarrhea. Fifty-six stools were Campylobacter positive according to the composite reference (44 by culture and 12 by ELISA and PCR). Among them, 42 were positive with the 2 ICTs. The Ridaquick detected 47 positive specimens whereas the ImmunoCardSTAT!Campy detected 44, corresponding to a sensitivity of 85.4% and 78.5% and a positive predictive value of 94% and 84.6%, respectively. Among the 249 composite reference negatives, 10 were positive with the ICTs: 7 with Immunocard and 2 with Ridaquick, i.e. a specificity of 97.1% and 99.1% and negative predictive values of 92.3% and 94.9%, respectively.

The performances of the ICTs appear to be satisfactory and allow a very rapid detection of Campylobacter which is important to treat early Campylobacter infection with an adapted antibiotherapy.

Detection of *Campylobacter* in water

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Contaminated water has been shown to be an important source of infection with *Campylobacter* for both broilers and humans. For epidemiological surveillance, more sensitive and reliable detection methods are needed. The number of *Campylobacter* in contaminated water is usually less than 1 CFU/L, and this puts great demand on the detection method. Large volumes of water are usually needed for reliable analysis. Many detection methods (e.g. ISO 17995 and NMKL 119) analyze only 1 L of water.

A method that involves filtration of large volumes (> 50 L) of water that are flushed or pumped through a dialysis filter (ultrafiltration) has been tested in this study. The filter (Asahi Kasei REXEED-25A) is composed of hollow fiber bundles with a total membrane surface area of 2.5 m^2. The filter concentrates everything in the sample that is greater than 30 kDa. In the experiments 60 L of autoclaved tap water per sample were spiked with low levels of *Campylobacter jejuni* or *C. coli* (0.1 – 330 CFU/60 L) in order to determine the limit of detection. The water samples were pumped through the filter and after the filtration the concentrated material in the filter was eluted through so-called "Backflush" system where the flow is reversed and 500 ml of elution buffer is pumped through the system. The *Campylobacter* in the eluate was then cultured by enrichment in 500 ml Bolton broth followed by plating on mCCDA.

Levels down to 10 CFU/60 L water sample were easily detected with this method. *Campylobacter* was occasionally detected in samples spiked with as few as 3-4 CFU/60 L water but this seems to be close to the detection limit.

These results indicate that ultrafiltration is a sensitive technique enabling concentration of low numbers of *Campylobacter* from large volumes of water.

Comparison of testing methodologies for enumerating levels of *Campylobacter* species from fresh whole raw chicken carcasses

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Background: Chicken meat has been incriminated as the key food-borne transmission route for *Campylobacter* infection. Current monitoring often involves examining a skin sample (neck-skin) but as insufficient may be present, investigation of alternative samples were evaluated based on sampling feasibility and comparability of results.

Objective: To compare sampling feasibility and testing outcomes for carcass-rinse, back-skin and neck-skin samples from chicken carcasses.

Methods: Fresh whole raw chickens (n=416) were tested. The level of *Campylobacter* cfu in the three sample types were compared using log10 cfu *Campylobacter* per g skin and per ml rinse as outlined in EC ISO/TS 10272-2 (2006). The detection limit was 10cfu/g.

Results: At least one of the three samples had *Campylobacter* isolated from 288 (69%) chicken carcasses. *Campylobacter* were detected in 185 chickens across the three sample types, but were not detected in any sample for 128. *Campylobacter* were detected in 208 neck-skin (50%), 218 back-skin (52%) and 280 (67%) carcass-rinse. Carcass rinse samples resulted in significantly more chickens testing *Campylobacter*. There was a reasonable agreement between counts for back, neck-skin and carcass-rinse samples. The percentage of samples with >1000 cfu/g (or ml) was 5.0 % (95% CI: 3.2-7.6%) for neck-skin samples, 2.9 % (95% CI: 1.5-5.0%) for back-skin samples and 5.3 % (95% CI: 3.3-7.9%) for carcass-rinse samples.

Conclusions: While all sample types provided satisfactory results neck-skin samples were identified to provide the most feasible and reliably comparative results. Reducing the amount of sample from 25 to 10 g neck-skin did not significantly hamper sample comparison.

Parallel session
« Poultry and non-poultry epidemiology and ecology of *Campylobacter sp.* »

Chairpersons:
DE ZUTTER Lieven, Belgium and DENIS Martine, France

Studying *Campylobacter jejuni*
in the chicken reservoir in Italy

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Broiler chicken is the main reservoir of *Campylobacter jejuni* making it the principal source of human campylobacteriosis. Despite the international importance of Italian poultry production, few studies focused on the characterization of *Campylobacter* in broilers and retail chicken meat. Here we describe the *C. jejuni* population in farms and foods from Italy. During the period 2015-2016, 527 *C. jejuni* strains were isolated at the abattoir and the retail market. Genomes were sequenced with Illumina technology and assembled de novo with SPAdes 3.8.1. MLST and cgMLST typing were performed using Ridom 3.2.1. Slaughter batches had a high prevalence of *C. jejuni*, which was significantly reduced in food. *C. jejuni* population circulating in Italian broiler farms included 90 sequence types (STs) and 22 clonal complexes (CCs). Strains isolated from retail meat belonged to 49 STs and 16 CCs. The most frequent types were ST-2116 (ST-353 CC) and ST-2863 (ST-354 CC), representing 20% and 10% of the total isolates, respectively. Despite the absence of reported *C. jejuni* ST-2116 from non-Italian poultry, these types have been isolated from human samples from other European countries. We further focused on the ST-2116 population through comparative genomic analysis including genomes of strains isolated from same farms in 2011 and a human sample from the UK. Little genetic diversity was observed across time, suggesting the recent expansion of a clone. Moreover, the high similarity between the British human and the Italian poultry isolates shows the potential of cgMLST analysis in identifying possible cross-border transmission, being more decisive than traditional methods for molecular epidemiology.
Our study makes clear the *C. jejuni* population circulating in Italian broiler farms and retail chicken suggesting the presence of two geographically restricted clones to devote more targeted interventions aimed at the prevention of disease.

Molecular characterization of *Campylobacter* from the Canadian Poultry Baseline Survey

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Campylobacter in broiler chicken was identified as a priority hazard-meat combination in Canada's Pathogen Reduction Initiative. Poultry is an important reservoir of *Campylobacter* and known to be major source of human exposure. Characterization of isolates collected at different production stages will provide a better understanding of prevalence and diversity of strains circulating in the Canadian poultry supply chain. We report here the results of a one-year national microbiological baseline study (MBS) in broiler chicken conducted by the Canadian Food Inspection Agency in collaboration with industry and federal, provincial and territorial partners.

Campylobacter isolates (n=2,458) were obtained from caeca samples, abattoir whole carcasses and parts at abattoir and retail raw chicken meat products. Isolates were subtyped by Comparative Genomic Fingerprinting (CGF); subtypes were compared to the Canadian Campylobacter CGF database (C3GFdb) to explore clinical associations, host specificity, and geographical distribution of poultry subtypes.

Although the *Campylobacter* population in Canadian broiler chicken was highly diverse (368 CGF subtypes), with nearly 48% of subtypes observed in a single instance. However, a small number of dominant CGF subtypes were observed, with the 20 most frequently observed subtypes accounting for 52% of isolates recovered in the study. As expected, study isolates were primarily from subtypes that have been primarily associated with poultry. However, although 80% of study isolates were from subtypes representing 42% of human clinical isolates in the C3GFdb, a small number of subtypes (n=6) highly prevalent among campylobacteriosis cases also have a strong source association to cattle.

Results from the MBS are consistent with studies that show that poultry plays a leading role in campylobacteriosis. At the same time, our results highlight the potential contribution of non-poultry-associated sources of *Campylobacter* to the epidemiology of campylobacteriosis.

Characterization of *C. jejuni* isolates from game birds in Finland

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Relatively little is known about *C. jejuni* genotypes found in different bird species and their environments, especially game birds. We isolated *C. jejuni* from mallard ducks (*Anas platyrhynchos*, n=100) in 2014 and pheasants (*Phasianus colchicus*, n=100) in 2013 during the hunting season in Southern Finland. The birds were plucked (mallard duck) or skinned (pheasant) and eviscerated immediately after hunting and intestines were transported chilled to the laboratory. Fecal samples were cultivated either directly or after enrichment on mCCDA or alternatively by selective filtration (0,65 µm) of a 1:10 dilution of the fecal sample on Nutrient blood agar. Ten (10%) samples from pheasants and 76 (76%) from mallard ducks were positive for *C. jejuni*. All *C. jejuni* isolates from pheasants were subjected to whole genome sequencing. For the mallard duck isolates, PFGE was first used to screen the genomic variability among the isolates and 35 strains representing all the different PFGE profiles (some in duplicate) were selected for whole genome sequencing. Altogether 16 different sequence types (STs) were identified four of which were novel to the PubMLST database. By taking into account the combined results of PFGE and MLST the most common STs in mallard ducks were ST-2314 (n=23), ST-1299 (n=16), ST-991 (n=6) and ST-2839 (n=5). All these STs have previously been reported in the Campylobacter PubMLST database also from human stool samples (except for ST-2839, source unknown). Pheasant samples were collected on three separate occasions and from two different locations, however, all the isolates represented ST-19 (ST-21 clonal complex), which has previously been isolated from patients (gastroenteritis, systemic disease and Guillain Barré syndrome Syndrome) as well as a wide variety of different carriers worldwide.

In conclusion game birds may pose a risk for acquiring campylobacteriosis and should be handled accordingly. More detailed genome-wide characterization of the isolates will be presented.

Diversity of *Campylobacter lari* in a One-Health context:
Focus on shellfish, wild birds, surface water and human health risk

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The microbiological quality of coastal environments can be affected by fecal pollution from urban and agricultural sources and by wildlife. Within these settings, shellfish for human consumption is impacted by microbial contamination. *Campylobacter lari* is the most frequently detected *Campylobacter* species in shellfish and sporadically causing human gastroenteritis. While seabirds are a potential source of shellfish contamination, the occurrence of *C. lari* in the wider environmental water cycle is poorly documented.

The aims of this study were to evaluate the occurrence of *C. lari* in a shellfish-harvesting area and its livestock farming-intensive catchment in Brittany, France. The diversity and distribution of *C. lari* isolates was assessed in mussels, oysters, cockles, wild birds, surface freshwater, seawater and human feces samples. In total, more than 300 *C. lari* isolates underwent comparative analysis by whole genome sequencing (WGS) on Illumina MiSeq. Genome assembly and MLST genotyping were performed using SeqSphere+ (Ridom).

While *C. jejuni* and *C. coli* were the most frequently isolated species in river waters (35.5% and 55.7%, respectively), *C. lari* was predominant in shellfish (90.2% species dominance), seabirds (56.9%) and marine waters (54.5%). WGS analyses of *C. lari* revealed that 80% of isolates had putatively novel sequence patterns. Shellfish isolates tended to preferentially cluster with seabird isolates, whereas strains from the upstream catchment were different. These findings show a hitherto unreported high diversity of *C. lari*, and suggest distinct adaptive behavior and ecological sources or reservoirs of *C. lari* in shellfish.

Future WGS analyses, including core genome MLST and the detection of virulence genes, will be useful to document *C. lari* throughout the environmental water cycle as well as the link to human feces isolates.

A large survey on *Campylobacter* contamination in bovine production in France

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Campylobacter is the leading cause of bacterial gastroenteritis in industrialized countries, with poultry reservoir as the main source of infection. Nevertheless, a recent study on source attribution has shown that the bovine reservoir could be a source of human contamination in France (Thépault *et al*., 2017). However, few data are available on bovine contamination in France. The aim of this project is to collect new data and to subtype *Campylobacter* strains to be included in the current French source attribution study.

A 6-month survey was carried out in one of the largest European bovine slaughterhouse. A statistical representative sampling plan was designed on the basis of previous data in France (16.5%; Châtre *et al*., 2010). Thus, 959 intestinal samples (493 beef cattle and 466 calves) were collected. *Campylobacter* were detected according to the EN.ISO.10272 method and *Campylobacter* species were identified by Maldi-Tof. More than 2000 *C. jejuni* isolates have been collected and a selection of 650 isolates are being typed by Comparative Genomic Fingerprinting (CGF40).

The estimation of total *Campylobacter* spp. prevalence was 69.1%; within the beef cattle samples, the prevalence was 40.6 % while 99.4% of calves' samples were contaminated. *C. jejuni* was the species the most prevalent with 38.6% and 98.5% in beef cattle and calves samples, respectively. The prevalence of *C. coli* was lower with 3% and 12.5% in beef cattle and calves samples, respectively. The first results of CGF40 typing showed a high genetic diversity with new molecular patterns.

This first large investigation allowed the collection of new data in the bovine reservoir which enabled the estimation of *Campylobacter* prevalence in beef cattle and calves. Indeed, these animals showed to be highly contaminated by *C. jejuni* especially calves. The results of this investigation are valuable for the source attribution of campylobacteriosis in France.

Parallel session
« Antibiotics and antimicrobial resistance »

Chairpersons:

LEHOURS Philippe, France and KEMPF Isabelle, France

ST5136 is the largest vertically expanding UK wide clone of *Campylobacter jejuni*.

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Introduction: *Campylobacter jejuni* and *Campylobacter coli* are the most common causes of bacterial gastroenteritis in the developed world. The aim of this study was to understand how antibiotics in the farm environment select for antibiotic resistant clones of *Campylobacter*. The diversity of tetracycline resistance determinants and quinolone resistances conferred by *gyrA* mutations in *Campylobacter* isolated form different host reservoirs was assessed.

Materials and methods: DNA from pure cultures of *Campylobacter* spp. from chicken, cattle, sheep and humans in Grampian region of Scotland was extracted and whole genome sequenced using an Illumina HiSeq 2000 sequencer with 100 base paired-end sequencing and the FASTQ paired-end reads assembled using Velvet. The genomes were assessed for purity and submitted to the Bacterial Isolate Genome Sequence Database (BIGSdb) where alleles were tagged and the presence of the tetracycline resistance determinant CAMP1698 and the C257T *gyrA* mutation identified.

Results: Whole genome MLST analysis using 136 representative strains isolated from UK identified that ST5136 is a UK wide clone, having emerged from ST464 through substantial genetic recombination. ST5136 was exclusively associated with chicken, turkey and humans and was the most prevalent strain harbouring the *tet*(O/32/O) $_{7\text{-like}}$ determinant. The most common tetracycline resistant alleles were *tet*(O/32/O)$_7$, *tet*(O/32/O)$_{8,\ 13}$. The *tet*(O/32/O) $_7$ variant was chromosomally associated in ST5136. 99% (207/209) of CC464 strains were ciprofloxacin resistant and had the C257T *gyrA* mutation.

Conclusion: The *tet*(O) [Φ-m46.1] and *tet*(O)-like variants in *Campylobacter* were closely related. The *tet*(O/32/O) from human gut bacteria and *Campylobacter* were also similar in sequence suggesting the potential for *Campylobacter* and other species to rapidly evolve through common DNA transfer systems. The increase in resistance to ciprofloxacin and tetracycline in clonally expanding populations of *Campylobacter* and the usage of these antibiotics in agriculture is discussed.

Emerging antibiotic resistance in ruminant *Campylobacter* in the United States

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Ruminant *Campylobacter* contributes significantly to outbreaks and sporadic cases of foodborne illnesses in humans. Antibiotics such as fluoroquinolone (FQ) and florfenicol are frequently used in cattle for disease prevention and control in the U.S., but little is known about antibiotic resistance in bovine *Campylobacter.* To facilitate the control of antibiotic-resistant *Campylobacter*, we analyzed *C. jejuni* and *C. coli* isolates obtained from 35 feedlot cattle farms in multiple states. The results revealed high prevalence of FQ resistance: 35.4% in *C. jejuni* and 74.4% in *C. coli*. While most FQ-resistant *Campylobacter* isolates harbored resistance-conferring mutations in GyrA, some of the FQ-resistant isolates did not have any known mutations in GyrA, suggesting the presence of unknown mechanisms for FQ resistance. Molecular typing of FQ-resistant isolates further revealed that clonal expansion was involved in dissemination of FQ-resistant *C. coli* but not *C. jejuni*. Notably, florfenicol resistance, which was historically low in *Campylobacter*, also emerged in the bovine *Campylobacter* isolates. Whole genome sequencing analysis identified a novel *cfr* variant, *cfr(C)*, in the florfenicol-resistant isolates. The Cfr(C) ORF is divergent from Cfr and only shares 55.1% and 54.9% amino acid identity to Cfr and Cfr(B), respectively. Cloning of *cfr (C)* into *C. jejuni* NCTC11168 and conjugative transfer of the *cfr (C)*-containing plasmid confirmed its role in conferring resistance to multiple classes of antibiotics including phenicols, lincosamides, pleuromutilins, and oxazolidinones. The *cfr(C)* gene was detected in 10% of the *C. coli* isolates, and molecular typing of the *cfr(C)*-positive *C. coli* isolates revealed its spread mainly via clonal expansion. These findings reveal the rising prevalence of FQ-resistant *Campylobacter* and the emergence of a novel multidrug resistant mechanism Cfr(C) in ruminant *Campylobacter* in the U.S. Acquisition of these resistance traits likely facilitates *Campylobacter* adaptation to the selection pressure from antibiotic usage in cattle.

Antimicrobial resistance in *Helicobacter pylori* – a 7 year perspective from Gastrointestinal Bacteria Reference Unit (GBRU), Public Health England

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Objectives: Macrolide resistant *Helicobacter pylori* was recently been included in the WHO list of "priority pathogens". National Guidelines for England and Wales recommend performing gastric biopsies for *H. pylori* culture and antimicrobial susceptibility testing for patients with dyspepsia who have failed first- and second-line treatment, have limited options due to drug hypersensitivity or live in an area with a high local resistance rate. We sought to determine antimicrobial resistance rates of *H. pylori* referrals to Gastrointestinal Bacteria Reference Unit (GBRU) over the last 7 years.

Methods: All gastric biopsies and *H. pylori* isolates referred to GBRU from January 2011 to December 2016, were analysed. Culture for *H. pylori* was undertaken for all gastric biopsies. *H. pylori* isolates underwent phenotypic susceptibility testing to using disc diffusion and E-tests. Data were analysed using Excel 2010.

Results: 4430 clinical specimens (gastric biopsies and isolates) from 3767 patients were tested by GBRU over a seven-year period (2011-2016), *H. pylori was* cultured in 1541 (34.7%). Phenotypic resistance (as % of culture positive specimens) was as follows: amoxicillin 4.0% (61), clarithromycin 72.7% (1121), levofloxacin 17.6% (271), metronidazole 86.4% (1331), rifampicin 13.7% (211), rifabutin 0.3% (5) and tetracycline 1.7% (26). Multidrug resistance (defined as resistance to ≥2 antimicrobial drugs) was found in 73.8% (1137) and mono-drug resistance in 18.4% (283). Resistance to ≥3 antimicrobial drugs was found in 24.8% (382). There were none identified with pan resistance (7 antibiotics) with eight resistant to 5 drugs and one resistant to 6 drugs (tetracycline, amoxicillin, clarithromycin, metronidazole, levofloxacin and rifampicin).

Conclusions: Our data suggest that secondary antimicrobial resistance rates of *H. pylori* in difficult-to-treat dyspepsia are high in England and Wales. Urgent surveillance is needed to determine the incidence of primary resistance and risk factors for developing antimicrobial resistance in such patients to guide treatment and prevention strategies.

Targeting *Campylobacter jejuni* lytic transglycosylase for drug discovery

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Production of β-lactamase is a major and threatening β-lactam resistance mechanism. To counteract β-lactam resistance, discovery of β-lactamase inhibitors has been the major emphasis in the past decades but has only led to partial success. Given the direct link between β-lactamase induction and cell wall metabolism in Gram-negative bacteria, inhibiting the induction of β-lactamase is a promising therapeutic strategy to counteract β-lactam resistance. Our recent studies have shown *Campylobacter jejuni* is a great model organism to test this hypothesis. In particular, the periplasmic lytic transglycosylase (LT) plays a critical role in regulating β-lactamase mediated β-lactam resistance in *C. jejuni*. In this study, we observed that the LT inhibitor bulgecin A inhibited the activity of the *C. jejuni* LT and significantly potentiated β-lactam antibiotic against resistant *C. jejuni*. To further develop LT inhibitor for combination therapy, we obtained the 2.16Å crystal structure of the *C. jejuni* LT; the fully refined structure revealed an interesting doughnut shape which is similar to the previously determined structures of *E. coli* SLT70 and *P. aeruginosa* MltE. It is remarkable that the doughnut-shaped feature is well maintained as *C. jejuni* LT is only 541 aa residues whereas Slt70 contains 618 residues. The complex structure of the *C. jejuni* LT with bulgecin A was also obtained, revealing active site residues important for LT activity. The active site of the *C. jejuni* LT situated in the catalytic domain is remarkably similar to that of *E. coli* Slt70 despite the only ~26% sequence identity. Together, this study strongly suggested that the soluble *C. jejuni* LT serves as an ideal representative target to perform LT-based drug discovery, and established a solid foundation for us to identify new lead compounds inhibiting LT using computational docking and high-throughput screening approaches in the future.

Plenary session
« Pathogenicity and virulence factors »

Chairpersons:
KOROLIK Victoria, Australia and MEGRAUD Francis, France

Nuclear remodeling in response to the cytolethal distending toxin: formation of nucleoplasmic reticulum

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Cytolethal distending toxin is a widespread secreted-toxin among Gram-negative bacteria. In the present study, we showed that CDT triggers the formation of nuclear invaginations continuous with the endoplasmic reticulum that enclose a cytoplasmic core. These structures, also called nucleoplasmic reticulum, were observed *in vivo* and *in vitro* and were particularly important in hepatocytes. They are composed of long tubular channels formed by invaginations of the nuclear envelope, which extend deeply into the nucleoplasm. They contain proteins involved in mRNA translation, polyadenylated RNA and ribosomes. The most important invaginations correlated with giant nuclei having a high degree of ploidy. All these features were not observed during infection with CDT isogenic mutant strains and with other non-CDT toxinogenic bacteria associated to profound reorganization of the cell actin cytoskeleton and stress fiber formation, such as Helicobacter pylori CagA+. The induction of the nuclear invaginations was attributed to the CdtB subunit of the CDT, with a determining role of the histidine residue at position 265 involved in catalytic activity. These CDT-induced invaginations were less important in the presence pharmacological inhibitor targeting the phosphoinositide 3-kinase. These data suggest that CDT induces the presence of ribonucleoprotein particles clustered and invaginated within the nucleus. Such a structure has never been reported during bacterial infections. Its importance in the pathologies associated with CDT remains to be defined, but its role could be associated with the maturation of some mRNAs, their post-transcriptional regulation, their preservation and/or storage in non-translated form.

Investigating *Campylobacter jejuni* interactions with endoplasmic reticulum in intestinal epithelial cells resulting in induction of the unfolded protein response.

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The Gram-negative bacterium *Campylobacter jejuni* interacts with, invades and survives within human intestinal epithelial cells (IECs). The endoplasmic reticulum (ER) is a critical organelle involved in multiple physiological functions, yet the extent of how *C. jejuni* interacts with the ER remains unknown. Our study has shown that *C. jejuni* interacts with the ER resulting in the activation of the unfolded protein response (UPR), a primitive, evolutionary conserved molecular signalling cascade that has been implicated in multiple biological phenomena including innate immunity and the virulence of bacterial pathogens including *Helicobacter pylori*, *Vibrio cholerae* and Shiga-toxigenic *Escherichia coli*. Our results also demonstrate that *C. jejuni* induces the UPR in a pathway-specific manner via the inositol-requiring 1α and X-box-binding protein 1 (IRE1α-XBP1) pathway. The *C. jejuni* cytolethal distending toxin (CDT) appears to have a role in inducing the UPR as both *cdtA* and *cdtB* mutants were significantly impaired in the induction of the IRE1α-XBP1 arm of the UPR. This study provides a new conceptual framework for further understanding the role of various *C. jejuni* virulence factors in the activation of UPRs and opens the way for understanding how *C. jejuni* diverges from the classical endocytic pathway within IECs, given that induction of the UPR by *C. jejuni* can lead to autophagic flux in IECs.

Identifying virulence factors that lead to abortion from zoonotic transmission and whole genome sequencing

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Campylobacter jejuni has emerged as an important cause of abortion in livestock, particularly small ruminants. This study investigated 17 *C. jejuni* isolates from clinical abortion cases from sheep, goats, and cows in seven Northern California counties. We hypothesized that a specific allele of as set of virulence genes accounted for the abortive phenotype. Each isolate was subjected to antibiotic resistance profiling, whole genome sequencing, and comparative genomics to determine the relationship between abortive and non-abortive genotypes. Using SMRT sequencing one isolate was sequenced to obtain the closed genome, the methylation density, and methyltransferase complement. The major modification motif was RAATTY with only m6A being found with 99% of the possible sites modified. We observed three distinctly different clades of abortive isolates with three divergent genotypes using genome-to-genome distance calculations. Nearly all isolates (16/17) contained *tetO*, the tetracycline resistance gene, in the chromosome or on a putative pTet –like plasmid. One isolate was multidrug resistant whose genome contained *tetO*, a SNP in *gyrA* that caused fluoroquinolone resistance, and *aphA*, an aminoglyscoside phosphotransferase conferring kanamycin resistance. It is reported that *porA* is responsible for abortion in model organisms. In this study, *porA* was divergent among abortive isolates and did not predict livestock abortion. Other adhesion factors (*jlpA*, *pebA*, and *cadF)* were associated livestock abortion, but were not allelically identical. The toxin gene, *cdtC*, was identical in every abortive isolate, but different as compared to non-abortive isolates. This study demonstrated that cdtC was predictive of livestock abortion.

Insights into *Campylobacter jejuni* colonization and enteritis using a novel infant rabbit model

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A lack of relevant disease models for *Campylobacter jejuni* has long been an obstacle to research into this common enteric pathogen. Here we used an infant rabbit to study *C. jejuni* infection, which enables us to define several previously unknown but key features of the organism. *C. jejuni* is capable of systemic invasion in the rabbit, and developed a diarrhea symptom that mimicked that observed in many human campylobacteriosis. The large intestine was the most consistently colonized site and produced intestinal inflammation, where specific cytokines were induced. Genes preferentially expressed during *C. jejuni* infection were screened, and *acs*, *cj1385*, *cj0259* seem to be responsible for *C. jejuni* invasion. Our results demonstrates that the infant rabbit can be used as an alternative experimental model for the study of diarrheagenic *Campylobacter* species and will be useful in exploring the pathogenesis of other related pathogens.

Gastric cancer-associated polymorphisms in the *Helicobacter pylori* type IV secretion system protein CagL: a potential biomarker for cancer risk

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The *Helicobacter pylori* CagL protein is essential for translocation of the oncogenic CagA protein into human cells. CagL is a key component of the *cag* pathogenicity island-encoded type IV secretion system. Previous studies suggest overrepresentation of particular polymorphisms within the 5-residue long CagL hypervariable motif (CagLHM) in *H. pylori* isolates from gastric cancer patients. However, these disease correlations were geographically variable and ambiguous. We compared the disease correlation of several hundred geographically diverse CagL sequences and identified >35 CagLHM sequence combinations with disparate geographical distribution, revealing substantial worldwide CagLHM diversity, particularly within Asian countries. Importantly, we discovered that certain CagLHM sequence polymorphisms correlate significantly with increased gastric cancer risks in *H. pylori*-infected patients. These associations were consistent across isolates from both Asian and non-Asian countries despite substantial sequence diversity of CagLHM across the globe. Thus, CagLHM regional diversity may contribute to the varied prevalence of *H. pylori*-related gastric cancer observed in diverse populations. These exciting findings point to the tantalising notion that CagL is a novel biomarker for early diagnosis of gastric cancer and a 'molecular beacon' for identifying key host factors and signaling pathways that underpin the pathogenesis of *H. pylori*-induced gastric cancer.

Helicobacter pylori expands and functionally activates a novel gastric stem cell population in a cag pathogenicity island-dependent manner

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The *H. pylori cag* pathogenicity island is a strain-specific constituent that induces epithelial responses with carcinogenic potential. Within the gastric epithelium, stem cells are critical for regulating self-renewal and maintaining tissue homeostasis. Lrig1 (Leucine-rich repeats and immunoglobulin-like domains 1) marks a distinct population of gastric stem cells. The aim of this study was to determine whether the *cag* pathogenicity island mediates Lrig1 stem cell activity and concomitant expansion within *H. pylori*-infected gastric mucosa. Lineage tracing was induced in six-week-old *Lrig1-CreERT2/+;R26R-YFP/+* mice; one week later, mice were infected with broth alone (control), the *cag*$^+$ *H. pylori* strain PMSS1, or a PMSS1 *cagE*$^-$ isogenic mutant, and sacrificed 2- and 8 weeks post-challenge. There were no differences in colonization efficiency or density between infected groups. Infection with wild-type PMSS1 for 8 weeks, but not 2 weeks, resulted in significantly ($p \leq 0.05$) increased inflammation and epithelial proliferation compared to uninfected controls or the *cagE*$^-$ mutant. Similarly, mice infected with wild-type *H. pylori* for 8 weeks, but not 2 weeks, harbored a significantly higher number of Lrig1-derived lineage traced glands, reflecting enhanced Lrig1 stem cell activity (uninfected=93 labeled glands, PMSS1=140 labeled glands, *cagE*=86.7 labeled glands; per 5□m section of gastric corpus, $p \leq 0.05$). Based on these findings, we subsequently generated gastric organoids and determined that, similar to *in vivo* findings, significantly ($p<0.05$) more organoids developed from mice infected with PMSS1 compared with controls or the *cagE*$^-$ mutant, reflecting increased stem cell activity. Using markers of metaplasia (GSII lectin) and surface mucus cells (UEA1) in combination with lineage tracing, we determined that Lrig1-traced cells generated metaplastic and surface cells. In conclusion, *H. pylori* induces expansion of Lrig1$^+$ cells and stimulates Lrig1 progenitor cell activity in a *cag*-dependent manner within an inflammatory milieu. These data identify *cag*-dependent signaling as a key mediator of epithelial stem cell function following infection by *H. pylori*.

Parallel session
« Control strategies for *Campylobacter* sp. »

Chairpersons:
HOFSHAGEN Merete, Norway, VAN DER LOGT Peter, New Zealand and CHEMALY Marianne, France

Relation between soiling of broilers and Campylobacter levels during the slaughtering process

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The Dutch poultry production sector is actively looking for ways to reduce *Campylobacter* levels on their products. Minimizing external fecal soiling of live birds could be a possible option to reduce *Campylobacter* contamination of chicken meat. Soiled flocks may be associated with higher levels of contamination from skin and feathers. Additionally, soiling may reflect intestinal disorders, leading to less uniformity and resulting in increased risk of contamination e.g. during evisceration.
The aim of this study was to determine the association - at flock level - between external fecal soiling of broilers upon arrival at the slaughterhouse and the number of *Campylobacter* colony forming units (CFU) on carcasses at three different stages of the slaughtering process. To examine whether the extent of soiling is of influence on *Campylobacter* levels during the slaughtering process (and ultimately on the end product), we tested two extreme categories; very clean birds against heavily soiled birds.
The categories were identified based on the footpad lesion score (0-200). This score is routinely recorded by Dutch slaughterhouses for broiler farms with animal densities over 39 kg/m². Foot pad lesions are associated with soiling of broilers as both are affected by litter quality. Flocks scoring <20 were considered 'clean' and flocks scoring >120 were considered 'soiled'.
A total of 22 *Campylobacter* positive flocks from a single slaughterhouse were included, 12 'clean' and 10 'soiled' flocks. From each flock, neck skin samples were taken after defeathering, pre and post chilling (5 birds per flock per location).
The median number of *Campylobacter* per gram neck skin varied between 3.0 and 3.6 log CFU for any of the three sample locations, with only small non-significant differences between clean and soiled broilers. It was concluded that soiling does not seem to increase the risk for *Campylobacter* contamination during the slaughtering process.

Isolation and development of *Campylobacater*-specific bacteriophages to combat bacterial colonization of animal hosts

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Campylobacter jejuni is a leading cause of bacterial-derived gastroenteritis, primarily due to the bacterium's ability to reside within the avian gastrointestinal tract. In the developed world, patients often become infected following consumption of undercooked poultry, while in the developing world, humans are often infected via contaminated water. Following ingestion, the bacterium adheres to the intestinal epithelium or mucus layer, where it causes toxin-mediated inhibition of fluid reabsorption from the intestine and invasion-induced inflammation and diarrhea. Additionally, *Campylobacter* is becoming increasingly resistant to antibiotics, including azithromycin and ciprofloxacin. To develop bacteriophage into a viable alternative to antibiotic treatment, we isolated viruses from multiple agricultural and environmental sources, including human wastewater and fecal samples from cattle, chickens, pigs, and sheep. *Campylobacter* loads were enumerated for each sample and bacteriophage were propagated, isolated, and purified from *C. jejuni* host cultures. Interestingly, no viable *Campylobacter* were isolated from chickens at a large processor, but bacteriophage were. To confirm this result, the microbiotas of these chickens were evaluated using 16s DNA analysis. Subsequently, using dose-response assays of purified phage, we identified the most potent bacteriophage against *C. jejuni* by evaluating pIC_{50} values. To determine the level of diversity, phage DNA was extracted and sequenced using Illumina NextSeq. Phage genomes were assembled and ordered into classes. These results indicate that diverse bacteriophage can be readily isolated from the environment, even in the absence of *Campylobacter*, and they may provide a specific, antibiotic-free method for reducing *Campylobacter* colonization of animal hosts.

A *campylobacter* putative outer membrane protein induces protective immune responses against an experimental *Campylobacter* infection in broilers

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Campylobacteriosis is the most prevalent human bacterial gastrointestinal disease in Europe. Birds are the main reservoir of *Campylobacter* and human contaminations principally occur by consumption and handling of poultry meat. It was estimated that a *Campylobacter* reduction from 2 to 3 $\log_{10}$ CFU/g of the intestinal contents in live broilers could be responsible for a decrease of 76% to 100% of the infection in humans. Vaccination of poultry may be a potential way in this goal but despite many studies, no efficient vaccine is available yet on the market and researches of more powerful vaccine antigens against *Campylobacter* are needed.

Fourteen potential new vaccine candidates against *Campylobacter* were recently identified using the reverse vaccinology strategy. Among these, a putative outer membrane protein never described before was selected to evaluate its protective immune response in broilers against experimental *campylobacter* infections. This vaccine antigen induced the production of specific IgY type antibodies in serum. More importantly, it induced also a mean reduction of caecal counts of 3.6 and 1.9 $\log_{10}$ CFU/g of caecal content in two individual experiments. This means that this antigen potentially can be used to significantly reduce the risk of transmission of *campylobacter* to humans. To achieve this goal additional experiments are needed to improve the vaccine efficacy and/or to develop a vaccine prototype to be used under field conditions.

Identification of potential Twin Arginine Translocation (TAT) pathways Inhibitors to control *Campylobacter* in poultry

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Campylobacter jejuni is a common cause of food poisoning in human worldwide. Despite the recognized significance of this pathogen in poultry, there is still a lack of effective and practical interventions to reduce human infections or to limit *C. jejuni* colonization in chickens. The Twin-arginine translocation (TAT) pathway is a critical feature of *C. jejuni* required for the commensal colonization of the chicken intestinal track and its response to stresses. Given mammals and chickens do not have proteins or receptors that are homologous to bacterial TAT proteins, the TAT pathway makes an ideal target for the development of specific antimicrobial therapies against *C. jejuni*. From over the 50,000 compounds screened at 6.25 µg, 779 molecules inhibited *C. jejuni* in a TAT-dependent copper sulphate growth inhibition assay. Based on *in silica* studies, 177 compounds with high druggable potential were selected for secondary screening. The combination of two TAT dependent assays (copper sensitivity assay and a formate dehydrogenase inhibition assay) identified 37 potential TAT dependent inhibitors. Eight compounds effectively inhibited the growth of *C. jejuni* strains of diverse genotypes and with no effect on commensal and beneficial gut microbes at 6.25 µg of compounds. Six of the eight compounds possessed low cytotoxicity at 50 µg on human intestinal epithelial cells (Caco-2 cells) and four molecules eradicated internalized *C. jejuni* in Caco-2 cells when treated with 2.5 µg of compounds. Further, three molecules resulted in reduction of up to 1.2-log *C. jejuni* population in ceca of broiler chickens with a minimum effect on cecal microbiota. Our future studies on compound derivatization and water solubility would enable the development of novel control method against *Campylobacter* in an industrial setting.

The *in vitro* and *in vivo* effect of Carvacrol in preventing *Campylobacter* infection, colonisation and improve chicken broilers productivity

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The current trend in reducing the antibiotic usage in animal production imposes urgency in identification of novel biocides, such as essential oils, effective against bacterial pathogens in a farm setup. Carvacrol, for instance, changes the morphology of the cell and acts against a variety of targets within the bacterial membranes and cytoplasm and our *in vitro* results show that it reduces adhesion, invasion of chicken intestinal primary cells and also biofilm formation. A trial was conducted to evaluate the effects of dietary supplementation of carvacrol at 4 levels (0, 120, 200, and 400 mg/kg of diet) on *Lactobacillus* spp., *E. coli*, *Campylobacter* spp. and broilers performance. Each of the 4 diets was fed to 3 replicates / trial of 50 chicks each from day 0 to 35. Our results show that carvacrol linearly decreased feed intake, feed conversion rates (FCR) and increased body weight (BW) at all levels of supplementation. Plate count analysis showed that *Campylobacter* was only detected at 35 days in the treatment groups compared with the control group where the colonisation occurred at 21 days. The absence of *Campylobacter* at 21 days in the treatment groups was associated with a significant increase in the relative abundance of *Lactobacillus* spp. Also, carvacrol was proven to have a significant decreasing effect on *Escherichia coli* numbers in the caecum of the treatment groups, at all supplementation levels.
In conclusion this study shows for the first time that at different concentrations carvacrol can delay *Campylobacter* colonisation of chicken broilers, by inducing changes in gut microflora, and places them as a promising alternative to antibiotics.

Parallel session
« Pathogenicity and virulence factors »

Chairpersons:
GAYNOR Erin, Canada and MEGRAUD Francis, France

The Ornithine Decarboxylase inhibitor Difluoromethylornithine effectively reduces *Helicobacter pylori* virulence and induction of inflammation and carcinogenesis

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Background: New targets for prevention of *H. pylori* (*Hp*)-associated gastric cancer are needed. We have shown that *Hp* induces ornithine decarboxylase (ODC), the rate-limiting enzyme for polyamine synthesis, and spermine oxidase (SMOX) that generates H_2O_2 from metabolism of spermine, generating oxidative stress-associated DNA damage. Inhibition of ODC with a-difluoromethylornithine (DFMO) or shRNA knockdown reduces *Hp*-induced DNA damage in gastric epithelial cells (GEC) *in vitro*. Our aim was to further investigate effects of DFMO in *Hp* infection.

Methods and Results: Mongolian gerbils were infected with *Hp* strain 7.13 for 8 or 12 weeks ± 1% DFMO in the drinking water. There was a significant reduction ($p<0.05$) in carcinoma with DFMO, associated with reduced polyamine levels. *Hp* output strains from DFMO-treated gerbils had decreased ability to translocate CagA into GEC versus isolates from untreated gerbils. Decreased CagA translocation correlated with rearrangements in *cagY*, a component of the Type 4 Secretion System, and sequencing revealed deletions in the *cagY* middle repeat region. *Hp* isolates from the DFMO-treated gerbils showed decreased capacity to activate NF-κB ($p<0.05$) in GECs using a luciferase reporter assay. IL-8 expression was decreased ($p<0.01$) in cells infected with *Hp* isolates from DFMO-treated versus untreated gerbils. Complementation of DFMO-output strains with *cagY* from parental 7.13 restored translocation of CagA and NF-κB activation, and complementation of 7.13 with *cagY* from DFMO strains had the reverse effect. Serial passage of *Hp* on plates containing DMFO, but not ornithine, also led to rearrangements in *cagY*, and reduced induction of NF-κB and IL-8.

Conclusions: Under conditions of polyamine depletion, carcinoma is reduced in gerbils infected with *Hp*. However, *Hp* isolates from DFMO-treated gerbils also showed rearrangements in *cagY*, associated with reduced CagA translocation and inflammatory responses. DFMO holds promise as a chemopreventive agent through beneficial affects on polyamine metabolism and attenuating virulence of *Hp*.

Further characterization of the ligand-binding properties of *Helicobacter pylori* sialic acid binding adhesin A (SabA)

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Helicobacter pylori is a highly persistent gastric pathogen in human and is a major causative agent of chronic gastritis and gastric cancer. Adhesion of *H. pylori* to gastric epithelial cells is an essential step leading to colonization and subsequently chronic infection. The *H. pylori* outer membrane protein SabA (sialic acid binding adhesin A) is crucial for the binding to inflamed gastric tissue through sialylated glyans e.g. sialyl lewis X (sLex). SabA consists of an N- terminal ecto-domain, a linker-region and a membrane-anchored β-barrel domain. The recently determined crystal structure of SabA ecto-domain (Pang *et al*., 2014) enables prediction of the putative ligand-binding pocket in the SabA ecto-domain. In this study, we generated isogenic *H. pylori* mutants with amino acid substitutions in the putative ligand-binding pocket and investigated their binding to sLex and laminin in comparison to that of the wild-type strain.

Investigating the biogenesis of *Campylobacter jejuni* outer membrane vesicle production

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Campylobacter jejuni constitutively releases outer membrane vesicles (OMV), although the exact process is unknown. Recently the *mla* pathway was implicated in a novel mechanism for the biogenesis of OMVs in Gram-negative bacteria. MlaA, a putative lipoprotein, and MlaC, a putative periplasmic binding protein, were found to impact vesiculation and maintain lipid asymmetry in the outer membrane. In this study we have investigated the role of the *mla* pathway in *C. jejuni* OMV biogenesis by comparing a *mlaA* mutant against the 11168 wild-type. Protein and lipid content associated with OMVs isolated from the mutant and complement strains was compared against 11168 wild-type OMVs. Protein content was quantified using a BCA assay and lipid content was quantified using a KDO assay. There were statistically significant increases in protein and lipid associated with OMVs from the *mlaA* mutant. To determine the phenotypic effect of the *mlaA* mutation, bacterial survival was investigated following incubation with sodium taurocholate (ST), lauryl sulfobetaine (LSB) and Polymyxin B. The *mlaA* mutant exhibited increased sensitivity to both Polymyxin B and ST induced stress. RT-PCR was used to determine expression of *mlaA* and *mlaC* in the absence and presence of 0.2% (w/v) ST and 0.5 mM LSB. *mlaA* and *mlaC* are both transcribed in the 11168 wild-type and expression was affected by either ST or LSB. Our results suggest MlaA and MlaC play a significant role in OMV biogenesis and maintaining the lipid asymmetry of the protective outer membrane.

Exploring the role of *Campylobacter coli* GT42 enzymes on lipooligosaccharides biosynthesis

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Sialic acid (*N*-acetylneuraminic acid, Neu5Ac) is the most naturally abundant nonulosonic acid. Sialylated glycoconjugates, which have been shown to influence pathogenesis through immune evasion, adhesion, and invasion, are synthesized by sialyltransferases. Functional and structural studies on *C. jejuni* LOS associated sialyltransferases (CstII and CstIII) have been carried out due to their association to the onset of the autoimmune Guillian-Barré syndrome. The CstII variants can either be monofunctional (α-2,3-sialyltransferase activity) or bifunctional sialyltransferases (α-2,3- and α-2,8- activity), while CstIII is monofunctional (α-2,3 activity). Although *C. coli* is the second most frequently isolated *Campylobacter* species, the activity of *C. coli* distant CstII homologues is unknown. Therefore, in the present study we explore the role of two *C. coli* putative sialyltransferases (CstIV and CstV) in LOS biosynthesis by combining genetic, mutational, recombinant protein, and LOS structural studies. Results show that despite several substitutions at amino acids deemed important for CstII activity, CstIV and CstV play an active role in LOS biosynthesis as deletion of *cstIV* and *cstV* result in LOS truncation. Nevertheless, no sialic acid has been detected in the intact LOS of *C. coli* strains expressing either CstIV or CstV by CE-MS and EA-OTLC-MS analysis. Furthermore, recombinant CstIV and CstV have not shown sialyltransferase activity on BODIPY-labelled acceptors so far. Nevertheless, LOS associated sialic acid biosynthesis gene (*neuB1*) appears to be involved in CstV substrate biosynthesis. Conversely, strains expressing CstIV lack *neuB1,* but contain orthologues involved in the biosynthesis of other nonulosonic acids, legionaminic (*neuB2*) and pseudoaminic acid (*neuB3*). Thus, their possible role in CstIV substrate synthesis is being investigated. Latest data showed that *neuB2* has no impact in LOS biosynthesis since insertion mutants and the wild type have identical LOS size. Finally, LOS structures synthesized by CstIV and CstV are currently being studied in detail.

TNFα affects the internalization of *Campylobacter jejuni* into human intestinal cells

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Campylobacter jejuni is a leading cause of foodborne bacterial enteritis worldwide. This pathogen was been described as an etiological factor in inflammatory bowel disease (IDB) in which pro-inflammatory cytokines, such as tumor necrosis factor α (TNFα) could play a major role. TNFα-stimulated intestinal epithelial cells have been used to initiate and to maintain mammalian cell inflammation. Inflammatory status was evaluated by the secretion of interleukin 8 (IL-8). Non mucus-secreting cells (HT29) and mucus-secreting cells (HT29-MTX) were used as virulence in vitro model. Inflammatory cells were obtained by TNFα-stimulation before adhesion and invasion assays. Among the 10 *C. jejuni* strains tested, the effect of TNFα pre-treatment on adhesion and internalization into eukaryotic cells was strain-dependent with the adhesion/invasion ability significantly altered in less than 50% of the strains. Interestingly, TNFα affects more strains in their ability to adhere and to invade the mucus-secreting HT29-MTX cells. In addition, the atypical aerotolerant *C. jejuni* Bf strain was the most invasive. This is the first study describing the behavior of *C. jejuni* in contact with TNFα-stimulated intestinal cells. Results suggest that the TNFα signaling pathway could participate in the internalization of *C. jejuni* in human intestinal cells in a strain dependent way. As some *C. jejuni* strains can behave differently on inflamed or non-inflamed epithelial cells, this in vitro virulence assay can provide preliminary data on the adverse effects caused by *C. jejuni* in patients suffering from a bowel inflammation, in order to determine whether such inflammation increases the risk of developing campylobacteriosis.

Plenary session
« Adaptation of *Campylobacter* sp. to environmental conditions»

Chairpersons:

KELLY Dave, United Kingdom, HADDAD Nabila, France

and Odile TRESSE, France

Poultry intestinal mucus modulates *Campylobacter jejuni* gene expression

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Campylobacter jejuni is an important human pathogen, causing up to 400 million infections a year world-wide. Poultry is a natural reservoir of *C. jejuni*, colonizing and residing within the intestinal mucus without causing disease. Mucus colonization is an essential step in *C. jejuni* colonization and previous studies suggest that host intestinal mucus impacts *Campylobacter* function. In this study we characterize the global transcriptome of *C. jejuni* grown on host mucus isolated from avian (chicken or turkey) and mammalian (cow, pig, or sheep) sources. *C. jejuni* NCTC 11168 was grown for 24 hours on defined media supplemented with or without 0.5 % wt/vol of each host mucus. Following RNA isolation, directional RNA libraries were sequenced, and mapped to the reference genome. Avian and mammalian mucus sources differentially impacted gene expression in ways that may reflect Campylobacter's ability to colonize different animal intestinal tracts. Non-coding antisense RNAs were associated with differentially expressed genes between avian and mammalian mucus, and may be in response to environmental cues. These data suggest that *C. jejuni* alters its gene expression in the presence of avian mucus in such a way that promotes intestinal colonization. Understanding how *C. jejuni* interacts within the host-intestinal environment will provide insights into how *C. jejuni* has adapted to colonizing different intestinal environments.

RNAseq reveals complex response of *Campylobacter jejuni* to bile and the ovine gallbladder environment

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Recent advances in the use of high throughput deep sequencing of RNA (RNAseq) have revolutionized the study of gene expression and have allowed unprecedented examination of the whole transcriptome of bacterial pathogens. The highly virulent *Campylobacter jejuni* sheep abortion (SA) clone, represented by the isolate IA3902, has recently emerged as the dominant cause for sheep abortion in the United States. The SA clone has also been increasingly identified from human outbreaks of foodborne gastroenteritis, making further understanding of the molecular mechanisms that allow for disease and persistence within the animal host of this particular strain is especially important. Survival of *C. jejuni* within the intestinal tract is known to require adaptation to various levels of bile salts. In addition, abattoir studies have frequently identified the gallbladder as a site of positive culture for *C. jejuni* in sheep despite the assumed inhospitable nature of this environment. To study the survival of *C. jejuni* IA3902 in bile and the ovine gallbladder, both *in vitro* and *in vivo* studies were performed to collect high quality total RNA following up to 24 hours exposure to these environments. High throughput deep sequencing of strand specific rRNA-depleted total RNA was then performed on the Illumina Hi-Seq platform to characterize the transcriptome of IA3902 and Rockhopper was used to analyze differences in gene expression. Our results indicated a large number of protein coding genes differentially expressed in ovine bile, the ovine gallbladder, and the ovine gallbladder mucosal layer along with differential expression of several previously identified small non-coding RNAs. This research provides valuable insight into the mechanisms that may be utilized by *C. jejuni* to survive and develop a carrier state within the inhospitable host gallbladder environment.

Contributions of Phase Variation to Phage-Escape by *Campylobacter jejuni*

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Background: Multiple surface-exposed structures of *Campylobacter jejuni* are subject to phase variation (PV) due to mutations in polyG/C tracts. Emerging structural PV-mediated differences in capsular polysaccharide (CPS) composition are linked to phage and serum resistance of *C. jejuni*. Specifically, ON/OFF switches in expression of CPS genes *cj1421*, *cj1422* and *cj1426* were shown to modulate sensitivity of NCTC11168 to phage F336 (Sorensen *et al.* 2011 J. Bact. 193:6742). Cj1421 and Cj1422 transfer O-methyl-phosphoramidate groups to alternate positions in CPS while Cj1426 attaches a 6-O-methyl group to the heptose. We hypothesize that this combination of hypermutable sequences and multiple phase-variable genes provides a fitness advantage to *C. jejuni* for survival of attacks by phage.

Method: We constructed deletion mutations of one, two or three genes for *cj1421*, *cj1422* and *cj1426* and complementation strains carrying *cj1421* with varying poly G tract lengths in *C. jejuni* strain NCTC11168 . Single strain and competition experiments of construct versus wild-type were performed in the presence and absence of phage. PolyG tract lengths were determined for multiple output colonies using a PCR-based-GeneScan method.

Results: The wild-type strain exhibited escape of phage due primarily to mutations in the polyG tracts of either *cj1421* or c*j1422*. Deletion of either of these genes did not produce changes in competitiveness but an increase in polyG tract length from G9 to G12 resulted in higher switching rates, enhanced escape of phage and increased competitiveness relative to the wild-type strain.

Discussion: Our study indicates that pre-existing variants generated by PV are key determinants of adaptation to selection pressures by *C. jejuni* and that the rate of switching can enhance competitiveness during selection. This study provides a framework for development of combined phage therapy and immunisation protocols targeting alternate phase-variable expression states of CPS epitopes as a strategy for reducing *C. jejuni* loads in poultry.

The phospholipidome of *C.jejuni*

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To survive, bacteria need to change not only their protein repertoire, but also their lipid composition in response to changes in their environment. Bacterial membranes are composed of phospholipids, glycolipids and proteins. In most cases, the phospholipids are composed of two fatty acids, a glycerol moiety, a phosphate group and a variable head group. Bacteria have evolved mechanisms to control the formation of fatty acids and modify the structure of existing fatty acids. These modifications allow bacteria to adjust their membrane viscosity to match environmental requirements. *Campylobacter jejuni* is a highly motile spiral shaped bacterium that is capable of changing its cell shape to a coccoid immotile bacterium. Knowledge of the composition of the phospholipids in the spiral or coccoid form of *C. jejuni* as well as the gene regulation and biosynthesis of the phospholipids is largely lacking. Moreover, changes in phospholipid composition in bacteria due to environmental conditions or age has hardly been studied in bacteria.

We followed the composition of the *C. jejuni* phospholipidome in cultures growing for 4 days on different carbon sources as well as oxygen availability by high performance liquid chromatography (LC-MS/MS) and studied the transcription of the genes involved in this process by RNA-seq. The phospholipidome of *C. jejuni* compromises more than a hundred different phospholipids and it displays a high variation dependent on the oxygen availability or age of the *Campylobacter* culture. Abnormal amounts (30-50%) of the phospholipids of *C. jejuni* are lysophospholipids. As in several pathogenic bacteria, accumulation of lysolipids is crucial to cause disease or to survive after phagocytosis, the role of the *C. jejuni* phospholipids in Campylobacteriosis might be an underestimated factor.

Membrane protein complexome of *C. jejuni* using 2D blue native/SDS-PAGE

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Campylobacter has emerged as the leading cause of bacterial foodborne infections in developed countries with a significant increase in the prevalence of campylobacteriosis cases for a decade. The perception cues from biotic or abiotic environments by the bacteria are often related to bacterial surface and membrane proteins. These proteins mediate the cellular response for the adaptation of *C. jejuni* to the environment. These proteins function rarely as a unique entity, they are often organized in functional complexes. In *C. jejuni*, these complexes are not fully identified and some of them remain unknown. To identify functional multi-subunit entities at the membrane subproteome level of *C. jejuni*, holistic non *a priori* method was addressed using two-dimensional (2-D) blue native (BN)/Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). Couples of acrylamide gradient/migration-time, membrane detergent concentration and hand-made strips were optimized to obtain reproducible extraction and separation of intact membrane protein complexes (MCPs). MCPs were subsequently denatured using SDS-PAGE and each spot from each MCP was identified by mass spectrometry (nanoLC-MS/MS). All together 20 MPCs could be identified including multihomooligomeric and multiheteroligomeric complexes. These MPCs are distributed in both inner and outer membranes. The maximum number of subunits detected in a MCP for *C. jejuni* using 2-D BN SDS-PAGE approach was three. Function and conservation of MPCs across *C. jejuni* strains were inspected by functional and genomic comparison analyses. The MPCs identified in *C. jejuni* membrane are involved in protein folding, molecules trafficking, oxidative phosphorylation, membrane structuration, peptidoglycan biosynthesis, motility and chemotaxis, stress signaling, efflux pumps and virulence. This is the first time that such a holistic non *a priori* method is applied to *C. jejuni* to detect MPCs.

Global control of *Campylobacter jejuni* biology by protein lysine acetylation

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The small genome size and regulator complement of *C. jejuni* suggests post-transcriptional adaptation mechanisms might be crucial in its life-cycle, but the control of protein activity by post-translational modifications (PTMs) of specific amino acids has not been widely studied. Here, we show for the first time that protein lysine acetylation is widespread in *C. jejuni* and that it impacts multiple aspects of its biology. Immunoblots using a commercially available anti-AcK antibody detected a wide range of acetylated proteins in strain NCTC 11168. While the acetylation profile was similar in both wild-type and *pta* mutants, deletion of *ackA* clearly increased protein acetylation, which returned to wild-type levels in a complemented strain. This pattern strongly suggests (i) a dominant role for chemical acetylation from acetyl-P, (ii) acetyl-CoA dependent acetylation via acetyl-transferases in the *pta* mutant. We identified a Sirtuin homologue, CobB which we show is a lysine deacetylase that is important for *in vitro* growth and *in vivo* colonisation of the *Galleria* larval model. Using a state-of-the-art proteomic workflow with antibody enrichment coupled to LC-MS/MS analysis, we have identified 7,322 acetylation sites in over 1,200 proteins in wild-type cells. Therefore, over 70% of the *C. jejuni* proteome is acetylated, a greater proportion than in any other bacterium to date. The *cobB* mutation caused significant alterations in the *C. jejuni* acetylome; we identified 566 lysines that are controlled by CobB and show that many fundamental cellular processes in *C. jejuni* are impacted by this reversible acetylation, including chemotaxis, motility, nitrogen and carbon metabolism. Target candidate proteins with cobB sensitive lysines were selected for further characterisation, to determine the effects of acetylation on their activity. Our results have revealed a previously unsuspected but extensive landscape of protein regulation by acetylation in *C. jejuni*, which we are now investigating in detail.

Parallel session
« Adaptation of *Campylobacter* sp. to environmental conditions »

Chairpersons:
KELLY Dave, United Kingdom and Odile TRESSE, France

The periplasmic methionine sulfoxide reductase system of *Campylobacter jejuni*: A new player in oxidative stress defence

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Oxidative stress is a major problem that *C. jejuni* must respond to for survival in the environment and growth in the host. Methionine residues are especially susceptible to oxidation by reactive chlorine species (RCS) at the sulfur atom to form methionine sulfoxide, a process reversed by Methionine Sulfoxide Reductases (MSR). Cytoplasmic MSR's are conserved throughout all life and are well studied, however it is only recently that a separate periplasmic Msr system has been identified in Gram-negative bacteria. In *C. jejuni*, this system is comprised of the soluble periplasmic reductase MsrP and its cognate inner membrane redox partner, MsrQ. We have used a mutagenesis and proteomics approach to identify the client proteins of MsrP, and show that methionine oxidation is directly responsible for client protein inactivation, and that this inactivation can be reversed by MsrP activity. We show that *msrPQ* expression is specifically regulated by RCS and propose a novel induction mechanism. We have also purified MsrP from *C. jejuni* and demonstrate catalysis *in vitro* using electrochemistry. Our results identify MsrPQ as a novel system to specifically protect vulnerable methionines in periplasmic proteins from oxidation.

The *Campylobacter jejuni* oxidative stress regulator RrpB is associated with a genomic hypervariable region and altered oxidative stress resistance

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Bacteria such as *C. jejuni* that can survive either within a host or in the environment require variable responses to survive the stresses associated with exposure to different levels of reactive oxygen species. The MarR-type transcriptional regulators RrpA and RrpB have recently been shown to play a role in controlling both the *C. jejuni* oxidative and aerobic stress responses. Analysis of 3,746 *Campylobacter jejuni* and 486 *Campylobacter coli* genome sequences showed that whilst *rrpA* is present in over 99% of *C. jejuni* strains, the presence of *rrpB* is restricted and appears to correlate with specific MLST clonal complexes (predominantly ST-21 and ST-61). *C. coli* strains in contrast lack both *rrpA* and *rrpB*. In *C. jejuni rrpB*$^{+}$ strains, the *rrpB* gene is located within a variable genomic region containing the IF subtype of the type I Restriction-Modification (*hsd*) system, whilst this variable genomic region in *C. jejuni rrpB*$^{-}$ strains contains the IAB subtype *hsd* system and not the *rrpB* gene. *C. jejuni rrpB*$^{-}$ strains exhibit greater resistance to peroxide and aerobic stress than *C. jejuni rrpB*$^{+}$ strains. Inactivation of *rrpA* resulted in increased sensitivity to peroxide stress in *rrpB*$^{+}$ strains, but not in *rrpB*$^{-}$ strains. The oxidative and aerobic stress responses of *rrpB*$^{-}$ and *rrpB*$^{+}$ strains suggest adaptation of *C. jejuni* within different hosts and niches that can be linked to specific MLST clonal complexes. More recently, we have begun investigating gene expression changes using RNA-Seq analysis between the *C. jejuni* 11168H wild-type strain and 11168H *rrpA* and *rrpB* mutants to obtain a global picture of expression changes in response to H_2O_2 stress. Combining this with EMSA and qPCR data, we aim to present a hypothetical model for the role of RrpA and RrpB in regulating *C. jejuni* responses to both oxidative and aerobic stress.

Campylobacter jejuni FliW modulates CsrA regulation of flagellar and non-flagellar protein expression via protein-protein interaction

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Introduction: Previously, we have shown that the *Campylobacter jejuni* post-transcriptional regulator CsrA is involved in biofilm formation, motility, interactions with host cells, and animal colonization. Functional flagella are required for each of these processes regulated by CsrA. This regulatory activity has been shown by our lab and others to be modified via protein-protein interaction with the flagellar chaperone protein FliW. We have determined protein regulatory events resulting from the loss of either CsrA or FliW. We have also begun mapping points of interaction between FliW and CsrA using a combination of deletion analysis and site directed mutagenesis.

Material and Methods: To identify the putative CsrA and FliW regulons using proteomics, wild type and mutants lacking CsrA or FliW were grown in parallel to mid-log and stationary phases. The relative abundance of whole cell proteins was determined using MS/MS, which allowed the identification of proteins dysregulated in the CsrA or FliW mutants. To determine the amino acid residues critical for FliW-CsrA binding, we generated deletion and point mutations using site directed mutagenesis of *C. jejuni* CsrA. The interaction of FliW with the various CsrA mutants was assessed using pull-down experiments, surface plasmon resonance, and bacterial 2-hybrid assays.

Results: Using proteomics experiments, we show that CsrA regulates 117 flagellar and non-flagellar proteins in both the mid-log and stationary growth phases. FliW regulates 153 proteins that heavily overlap those of the CsrA regulon, presumably by modulation of CsrA regulatory activity. Results indicate that the FliW binding site on CsrA is adjacent to one of the predicted RNA-binding interfaces on CsrA, suggesting a model by which FliW binding to CsrA destabilizes its RNA-binding ability and thus its regulatory activity.

Conclusion: Together, these demonstrate that CsrA regulates the growth-phase-dependent expression of both flagellar and non-flagellar proteins, and CsrA activity is controlled by binding to FliW.

Differential survival of hyper-aerotolerant *Campylobacter jejuni* under different gas conditions

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Campylobacter jejuni accounts for a significant number of foodborne illnesses around the world. *C. jejuni* is microaerophilic and typically does not survive efficiently in oxygen-rich conditions. We recently reported that hyper-aerotolerant (HAT) *C. jejuni* are highly prevalent in retail poultry meat. To assess the capabilities of HAT *C. jejuni* in foodborne transmission and infection, in this study, we investigated the prevalence of virulence genes in HAT *C. jejuni* and its viability in atmospheric, N_2 and CO_2 gas conditions. When we examined the prevalence of eight virulence genes in 70 *C. jejuni* strains from raw poultry meat, interestingly, the frequencies of detecting virulence genes were significantly higher in HAT *C. jejuni* strains than aerosenstive *C. jejuni* strains. Under aerobic conditions, aerosensitive *C. jejuni* survived at 4°C in raw poultry meat only a few days. However, HAT *C. jejuni* survived in poultry meat for a substantially extended time; there was a five-log CFU reduction over two weeks. N_2 and CO_2 demonstrated differential effects on the survival of HAT *C. jejuni*. Regardless of the aerotolerance level, N_2 marginally affected the viability of *C. jejuni*. However, CO_2 significantly reduced the viability of *C. jejuni* both in culture media and poultry meat.

These results suggest that HAT *C. jejuni* would potentially be more pathogenic than aerosensitive *C. jejuni*, and modified atmosphere packaging using CO_2 would probably help us to control HAT *C. jejuni* in poultry meat.

Characterisation of aerotolerant forms of a robust chicken colonizing *Campylobacter coli*

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Campylobacter coli OR12 is a robust coloniser of chickens that was previously shown to outcompete and displace other *Campylobacter* strains from the chicken's gastrointestinal tract. This strain is capable of aerobic growth on blood agar.

Serial aerobic passage increased this aerotolerance as assessed by quantitative assays for growth and survival on solid media. Aerotolerance was also associated with increased peroxide stress resistance. Aerobic passage did not alter cellular morphology or motility or hinder the microaerobic growth rate.

Colonisation of broiler chickens by aerotolerant *C. coli* OR12 was significantly lower than the wild-type strain at three days after challenge but not by seven days, suggesting adaptation had occurred. Bacteria recovered from chickens had retained their aerotolerance, indicating this trait is stable. Whole genome sequencing enabled comparison with the wild-type sequence. Twenty-three point mutations were present, none of which were in genes known to affect oxidative stress resistance. Insertions or deletions caused frame shifts in several genes including, phosphoglycerate kinase and the b subunit of pyruvate carboxylase that suggest modification of central and carbohydrate metabolism in response to aerobic growth. Other genes affected include those encoding putative carbonic anhydrase, motility accessory factor, filamentous haemagglutinin, and aminoacyl dipepdidase proteins.

Aerotolerance has the potential to affect environmental success and survival. Increased environmental survival outside of the host intestinal tract may allow opportunities for transmission between hosts. Resistance to oxidative stress may equate to increased virulence by virtue of reduced susceptibility to oxidative free radicals produced by host immune responses. Finally, resistance to ambient atmospheric oxygen may allow increased survival on chicken skin, and therefore constitutes an increased risk to public health.

Genomic and phenotypic characterization of *C. jejuni* water isolates

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Campylobacter can be transmitted via environmental pathways, such as untreated drinking water and recreational use of water. However, much is still unknown about the ability of *Campylobacter* to survive in water and the potential of water isolates to cause human infections.
We have here characterized seven *C. jejuni* strains isolated from raw (incoming) surface water at water plants in Sweden using whole genome sequencing (WGS), phenotypical assays and an *in vitro* infection model. Three strains belonged to ST clonal complex 48 (ST48CC), one to ST1275CC and three were unassigned. Phenotypical tests showed a lower motility for the ST1275CC strain compared to the other *C. jejuni* water strains. The strains belonging to ST48CC and ST1275CC together with one of the unassigned strains were able to form biofilm. Survival in water was tested over eight days in natural water collected at different time points during spring and fall from a Swedish lake. The ST48CC strains showed a higher survival in all water samples compared to the other water strains. All the *C. jejuni* water strains were able to adhere to and induce an IL-8 response in a human carcinoma cell line (HT-29) suggesting potential to cause infection. However, the levels of adherence and IL-8 response were lower for the water strains than for the *C. jejuni* reference strain NCTC 11168. Genomic analysis suggested some environmental adaptation, such as potential for arsenic resistance (*arsB*) and coding for one of two DMSO-reductases. Differences in presence of genes coding for putative virulence factors, such as CDT, Type VI secretions system and sialylated LOS, were identified.
In conclusion, the ability of all water strains to adhere to human cells and trigger a cellular response and the differences in water survival suggest that some *C. jejuni* strains may have more potential to cause waterborne infections.

Parallel session
« Immunology and Host response 2 »

Chairpersons:
ETHELBERG Steen, Denmark and FERRERO Richard, Australia

Differential gene expression, signaling pathways, and upstream regulator analysis of acute *Campylobacter jejuni* enteritis in human colon mucosa

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Introduction: We elucidated before that the diarrhea of *Campylobacter jejuni* (*C.j.*)-infected patients is based on two pathomechanisms, paracellular leak-flux and transcellular sodium malabsorption in the colon. Epithelial permeability in the human mucosa was increased for macromolecules, pointing to the *leaky gut* concept for immune activation. Now, we conducted RNA sequencing (RNA-Seq) in colon biopsies from these patients. Aim of this clinical observation study was to measure gene expression in patients' mucosae and to identify the predominant signaling pathways under the acute infection.

Methods: RNA from human colon biopsies was extracted with Trizol. cDNA library preparation and sequencing was performed on Illumina HiSeq2500. Demultiplexing was done with CASAVA pipeline. Reads were mapped against human genome GRCh37/hg19 using STAR. Further statistical analyses were performed by R software. Upstream regulators were identified by Ingenuity Pathway Analysis (IPA) software (Qiagen).

Results: In *C.j.*-infected human mucosa 2,988 transcripts were downregulated and 2,410 upregulated (Data are deposited in NCBI's GEO). By this gene expression data we determined the signaling pathways involved in *C.j.* infection by IPA. Upstream regulators are ranked upon their differentially expressed downstream target genes. The most significant upstream regulator with activating pathways was LPS (overlap P=3.22E^{-66}); second-most significant was IFN-gamma signaling (overlap P=3.95E^{-44}). Other top upstream regulators were also epithelial barrier-affecting TH1/TH2 cytokines like TNF-alpha or IL-13 (overlap P=3.23E^{-41} or P=1.39E^{-30}, respectively). On the other hand, the top upstream regulator with *inhibited* pathways was the active vitamin calcitriol (overlap P=8.97E^{-25}).

Conclusions: The disease transcriptome and upstream regulator data confirmed prior observations. Host RNA-Seq in *C.j.* infection provides a multitude of information along the *leaky gut* concept and bacterial genes should also be investigated in parallel. Specifically, the regulation pattern with inhibition of calcitriol-dependent pathways in the *C.j.*-infected mucosa led to the assumption that supplementation with vitamin D counter-regulates the inhibition of downstream targets.

Campylobacter jejuni infection of secondary abiotic mice lacking Nucleotide-Oligomerization-Domain-2

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Background: *Campylobacter jejuni* infections are rising worldwide. Given that innate immune receptors including Nucleotide-Oligomerization-Domain-2 (NOD2) are essentially involved in combating enteropathogenic infections, we here surveyed the impact of NOD2 in murine campylobacteriosis.

Methodology/Results: In order to overcome physiological colonization resistance preventing from *C. jejuni*-infection, we generated secondary abiotic NOD2-/- and wildtype (WT) mice by broad-spectrum antibiotic treatment. Mice were then perorally infected with *C. jejuni* strain 81-176 and could be stably colonized by the pathogen at high loads. Notably, NOD2 deficiency did not affect gastrointestinal colonization properties of *C. jejuni*. Despite high pathogenic intestinal burdens mice were virtually uncompromized and exhibited fecal blood in single cases only. At day 7 postinfection (p.i.) similar large intestinal histopathological changes and increases in numbers of colonic apoptotic cells could be observed in mice of either genotype, whereas *C. jejuni*-infected NOD2-/- mice displayed more distinct regenerative properties in the colon than WT controls. *C. jejuni* infection was accompanied by increases in innate and adaptive immune cells in mice of either genotype. Increases in T lymphocytes, however, were less pronounced in colons of NOD2-/- mice at day 7 p.i. when compared to WT mice, whereas colonic numbers of neutrophils and B lymphocytes were elevated in WT controls only. At day 7 p.i., colonic pro-inflammatory mediators increased more distinctly in NOD2-/- as compared to WT mice. Converse to the colon, however, ileal concentrations of nitric oxide, TNF, IFN-gamma, IL-6 and IL-10 were lower in NOD2-/- versus WT mice at day 7 p.i. Even though MUC2 was down-regulated in *C. jejuni*-infected NOD2-/- mice, this did not result in increased pathogenic translocation from the intestinal tract to extra-intestinal compartments.

Conclusion/Significance: In secondary abiotic mice, NOD2 signaling is required for the orchestra'ted host immune responses upon *C. jejuni* infection, but does not control pathogen loads in the gastrointestinal tract.

The role of Nod1 in targeting the immune response towards *H. pylori* in a genetic model of gastric carcinogenesis

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The *Helicobacter pylori* (*Hp*) *cag* type 4 secretion system (*cag*T4SS) is a cancer-linked locus which translocates CagA, DNA, and peptidoglycan into host cells. Nod1 is an innate immune receptor that recognizes and responds to peptidoglycan. We previously utilized a Mongolian gerbil cancer model to demonstrate that serial *Hp* infections induce oscillating patterns of inflammation; importantly, the development of cancer selected for attenuated *cag*T4SS virulence. However, gerbils are outbred, limiting the ability to study host constituents in pathogenesis. To define the role of Nod1 in this phenotype, we generated an inbred gastric cancer model with *Nod1* deficiency, INS-GAS-*Nod1*$^{-/-}$ mice, and infected animals with the *Hp cag*$^{+}$ strain PMSS1. Infected INS-GAS-*Nod1*$^{-/-}$ mice developed more severe inflammation ($p=0.0013$) compared with INS-GAS mice at 20 days post-challenge. However, this pattern was reversed during chronic infection (90 days) as *Hp*-infected INS-GAS mice developed more severe inflammation and a higher frequency of adenocarcinoma (80%) than INS-GAS-*Nod1*$^{-/-}$ mice (40%). To define underlying mechanisms, we quantified gastric mucosal cytokine profiles. Acute (2 days) infection of INS-GAS-*Nod1*$^{-/-}$ mice led to significantly increased levels of Th1/Th17 cytokines (IFNγ, IL-1α, IL-17, KC; $p<0.05$) compared to INS-GAS mice. To define the role of gastric epithelial cells (GEC) in regulating this acute immune response, we quantified cytokine secretion *in vitro* from primary mouse GEC co-cultured with the *Hp* strains PMSS1 and 7.13. Co-culture increased expression of KC and MIP-2 in *Nod1*$^{-/-}$ GEC compared to WT GEC. In contrast to the initial pro-inflammatory cytokine burst in *Nod1*$^{-/-}$ mice 2 days post-challenge, Treg/Th2 cytokine expression levels (IL-13, IL-10; $p<0.05$) increased at 20 and 90 days in conjunction with decreased levels of proinflammatory cytokines (IFNγ, IL-1α, IL-2, KC; $p<0.05$) in INS-GAS-*Nod1*$^{-/-}$ versus INS-GAS mice. These results indicate that Nod1 plays an instrumental role in regulating immune responses to *Hp* within the context of gastric carcinogenesis.

Helicobacter pylori modulates host cell responses by CagT4SS-dependent translocation of intermediate metabolites of inner core LPS biosynthesis

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Highly virulent *Helicobacter pylori* cause proinflammatory signaling inducing the transcriptional activation and secretion of cytokines such as IL-8 in epithelial cells. Responsible in part for this signalling is the *cag* pathogenicity island (*cag*PAI) that codetermines the risk for pathological sequelae of an *H. pylori* infection such as gastric cancer. The Cag type IV secretion system (CagT4SS) encoded on the *cag*PAI can translocate various molecules into cells. Previous studies have shown that the *cag*PAI transports the effector protein CagA, peptidoglycan metabolites and DNA. Although these transported molecules are known to contribute to some extent to cellular responses, a major part of the *cag*PAI-induced signaling leading to IL-8 secretion remained unexplained. Based on previous reports that LPS modifications might codetermine proinflammatory activity by *H. pylori*, we have approached the question by testing various fundamental LPS biosynthesis mutants for activation of human cells.

We report here that the biosynthesis pathway of heptose-1,7-bisphosphate, an important intermediate metabolite in LPS heptose inner core biosynthesis, contributes to the induction of proinflammatory signaling and IL-8 secretion by *H. pylori* in human epithelial cells, also dependent on an active *cag*PAI. We generated CRISPR Cas9 k/o cells in two different human cells lines and could thereby identify a cellular factor which is involved in the LPS heptose-phosphate pathway-induced responses by *H. pylori*. Two central enzymes in *H. pylori* were able to contribute to the biosynthesis of cell-activating LPS intermediates which we found to be present in *H. pylori* soluble preparations.

These novel results pave the way for a better understanding of *H. pylori*-induced signaling mediated by intracellular adaptors and pattern recognition receptors. They will also allow to better dissect immunomodulatory activities by *H. pylori* and to improve the possibilities of intervention in *cag*PAI- and inflammation-driven cancerogenesis.

The role of gelatinases
in *Campylobacter jejuni* infection
of secondary abiotic mice

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Background: Matrix metalloproteinases (MMP) -2 and -9 (also referred to gelatinases -A and -B, respectively) are upregulated in the inflamed gut of mice and men. We here addressed whether gelatinase were involved in mediating *C. jejuni* infection *in vivo*.

Methodology / Results: Secondary abiotic MMP-2-/-, MMP-9-/- and wildtype (WT) mice were generated by broad-spectrum antibiotic treatment and perorally infected with *C. jejuni* strain 81-167. The pathogen stably colonized the murine intestinal tract irrespective of the genotype, but did not translocate to extra-intestinal compartments. At days 8 and 14 postinfection (p.i.), less pronounced colonic histopathological changes were observed in infected MMP-2-/- mice, less distinct epithelial apoptosis, but more epithelial proliferation in both MMP-2-/- and MMP-9-/- mice, as compared to WT controls. Reduced immune responses in gelatinase-deficient mice were characterized by lower numbers of effector as well as innate and adaptive immune cells within the colonic mucosa and lamina propria. The expression of IL-22, IL-18, IL-17A and IL-1beta mRNA was higher in the colon of MMP-2-/- as compared to WT mice. Remarkably, synthetic gelatinase blockage reduced large intestinal pro-inflammatory immune responses and apoptosis in *C. jejuni* infected WT mice.

Conclusion: Both MMP-2 and MMP-9 are differentially involved in mediating *C. jejuni*-induced intestinal immunopathology. Pharmacological gelatinase blockage might be a promising option to ameliorate immunopathological sequelae of campylobacteriosis.

TRPM2 ion channel modulates macrophage function impacting gastric inflammation during *Helicobacter pylori* infection

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Helicobacter pylori infection of the gastric mucosa triggers a vigorous innate and adaptive immune response characterized by local increase of oxidative stress, and the accumulation of polymorphonuclear leukocytes, macrophages, and lymphocytes. While some inflammation is necessary to control the proliferation of *H. pylori,* inflammation and oxidative stress responses must be regulated to minimize pathology. Because of its role as an oxidative stress sensor in phagocytes, the function of the cation channel transient receptor potential melastatin 2 (TRPM2) was investigated during *H. pylori* infection. *Trpm2*$^{-}$*/*$^{-}$ mice, when chronically infected with *H. pylori*, exhibit increased gastric inflammation and decreased bacterial colonization compared with wild-type mice. The absence of Trpm2 promotes macrophage M1 polarization in response to *H. pylori* compared to wild-type macrophage responses. This *Trpm2-/-* hyper-M1 phenotype is accompanied by increased calcium overloading, enhanced MAPK activity, increased pro-inflammatory cytokine production and high ROS production. While the *Trpm2-/-* mice expressed higher levels of pro-inflammatory IL-1beta and IL-6, IL-17 expression was not enhanced and IFN-gamma was reduced compared to infected wild-type mice; Tregs were migrating into the tissues in greater numbers. Inhibition of the NADPH oxidase in *Trpm2-/-* macrophages reduced not only the ROS production but also reduced MAPK activation and cytokine production. While wild-type macrophages can be polarized *in vitro* to exhibit M1 or M2 characteristics, *Trpm2*$^{-}$*/*$^{-}$ macrophages never fully polarize to M2 macrophages suggesting Trpm2 activity may be important for wound healing and limiting inflammation. Ongoing experiments in the lab are investigating the links between Trpm2 and apoptosis, metabolism and M2 activation. These findings will help us understand how phagocytes regulate inflammation during *H. pylori* infection and this knowledge may benefit us when trying to enhance tissue repair processes in the gastrointestinal tract.

Plenary session
« Emerging and Related Organisms species »

Chairpersons:
HOUF Kurt , Belgium and CAPPELIER Jean Michel, France

Evolutionary genomics of gastric *Helicobacter* species revealed interspecies admixture and recombination and evidence for niche adaptation

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Since the description of the human pathogen *Helicobacter pylori*, various non-*H. pylori Helicobacter* species (NHPH) have been identified in the stomach of domesticated animals, marine mammals and wild felines. Most of these gastric NHPH have a pathogenic potential in their natural host and several are also of zoonotic importance. To better understand the genomic history of these ecologically similar but genetically distinct species, we carried out an evolutionary analysis of 107 gastric *Helicobacter* genomes. Additionally, 55 enterohepatic *Helicobacter* genomes were included for comparison. Population structure analysis established each gastric species into genetically differentiated groups with the exception of *H. baculiformis*, *H. cynogastricus* and *H. acinonychis* belonging to the *H. salomonis*, *H. felis* and *H. pylori* (in particular ancestral Africa) groups, respectively. Patterns of intraspecies admixture was evident within each population whereas interspecies admixture was observed between: *H. felis* and *H. cynogastricus* (34%); *H. baculiformis* and *H. salomonis* (16%); *H. cetorum* and *H. pylori* (ancestral Amerind) (21%); and *H. acinonychis* and *H. pylori* (ancestral Africa) (37%). FineSTRUCTURE analysis unravelled many events of genetic exchange within and across the canine and feline gastric *Helicobacter* spp., with *H. heilmannii* and *H. bizzozeronnii* showing the highest interspecies recombination. Genetic exchange between *H. pylori*, *H. acinonychis* and *H. cetorum* was also seen. Because these latter species don't share the same host, this phenomenon is most likely a remaining signal of ancestry.

Several genes overrepresented in the gastric species and underrepresented in the enterohepatic group encoded a function that could be assigned to the survival in the gastric niche of which some also underwent selection. Analysis of gene tree topologies even unravelled evidence of interspecific gene introgression. Our data showed new insights into the evolutionary adaptation of gastric *Helicobacter* species to the stomach with evidence of admixture and high interchangeability between species.

Importance of *C. rectus* CiaB to Host Cell Invasion and Host Response

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Introduction: *Campylobacter rectus* is an oral campylobacter that has been linked to periodontitis (gum disease). Additionally, *C. rectus* has been isolated from Barrett's esophagus, appendicitis, as well as oral and extraoral abscesses. Though not considered a highly pathogenic periodontal microbe, *C. rectus* has been implicated in bacteremia and has an established association with pre-term births and low birth weight indicating its importance as an emerging pathogen.

Methods: In order to investigate the potential virulence factors in this organism we have used allelic replacement to generate a series of mutant strains in putative secretion systems. In particular, we have focused on the campylobacter invasion antigen b (*ciaB*) gene as well as a flagellar secretion system (*flhB*) gene to gain insight into whether flagellar T3SS functions in *C. rectus* pathogenesis.

Results: We have validated the loss of *ciaB* expression in the mutant strain by real-time PCR and have established three *C. rectus* reference genes for real-time PCR. Q_PCR demonstrated no polar transcriptional effects for the *ciaB* deletion. We have also established that *ciaB* expression is critical for maximal invasion of host cells.
Following up on the the role CiaB plays in the host response to *C. rectus* we have assessed the inflammation response by utilizing a cell culture assay at 6 h time points under anaerobic conditions using a human placental epithelial cell line (BeWo). Post-bacterial exposure, BeWo RNA was extracted and inflammatory gene expression was observed using Bio-Rad's Human Gram-Negative Bacterial Infection PrimePCR assay plates.

Conclusions: This study suggests *C. rectus* uses CiaB to aid in the evasion of the host cell immune response and provides further evidence for its role in adverse pregnancy outcomes. Ongoing studies will test the importance of *C. rectus* CiaB to macrophage apoptosis.

Genome analysis reveals novel mobile genetic elements containing virulence-factor-like proteins in *Campylobacter concisus* strains isolated from patients with IBD

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Introduction: *Campylobacter concisus* is a Gram negative oral bacterium that is associated with human inflammatory bowel disease (IBD). Previous studies showed that some oral *C. concisus* strains have the potential to cause enteric diseases and they were suggested to be the initiators of human IBD. The aim of this study was to investigate the virulence factors of C*. concisus* that are associated with IBD.

Material and methods: Fifty *C. concisus* strains isolated from patients with IBD and controls were examined. PacBio genome sequencing, comparison of *C. concisus* genomes and detection of the expression of putative virulence factors using proteomics analysis were performed.

Results: A novel *C. concisus* mobile genetic element and a putative virulence factor were found to be associated with patients with active IBD.

Conclusion: This study provides novel information regarding *C. concisus* virulence.

Identification of a novel *Campylobacter* species in the gut microbiome of harbour seal pups (*Phoca vitulina*): *Campylobacter blaseri* sp. nov.

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We investigated the microbiome of 98 stranded harbour seal pups admitted for rehabilitation at the Sealcentre Pieterburen in the Netherlands using 16S amplicon sequencing before and during rehabilitation up until release. The microbiome of harbour seal pups was primarily composed of *Escherichia*, *Fusobacterium*, *Bacteroides*, *Psychrobacter*, *Anaerobiospirillum*, *Clostridium* and *Campylobacter* species. Interestingly, the 450 bp V3-V4 region of the 16S rDNA molecule of the *Campylobacter* species had no known representatives in the 16S databases and was only 95% identical to that of *C. gracilis*, *C. jejuni*, *C. upsaliensis* and *C. ureolyticus*.

This novel *Campylobacter* species is present in the microbiome of half of the seal pups and it is already present in seal pups upon arrival in the centre independent of their health status. Therefore, we conclude it's a normal inhabitant of the harbour seal gut. It appears to be overrepresented in older seals, both in prevalence and density. The density in the fecal microbiome is 2% on average, however, in several cases the density reaches 20% of the seal fecal microbiome.

The novel *Campylobacter* species was subsequently isolated from fecal samples, plated on Skirrow medium and incubated under microaerobic conditions. Phenotypically, it appears to be non-motile, urease positive, and shows growth at 25°C, which discerns it from all other currently known *Campylobacter* taxa. Phylogenetic analysis of the complete 16S region identifies the *Campylobacter* species as a distant relative of *C. ureolyticus*. Whole genome sequencing of several representatives of the novel *Campylobacter* species is ongoing and will be presented. The name *Campylobacter blaseri* sp. nov. is proposed for this new taxon, in honour of prof. dr. Martin Blaser.

Arcobacter: novel taxa in New Zealand shellfish

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The genus *Arcobacter* contains *Campylobacter*-like organisms originally recovered from human faeces, livestock, and the environment. In recent times, a range of new species have been recovered from marine environments, including shellfish. We investigated, for the first time, shellfish in New Zealand for such organisms. Isolation procedures described for recovery of marine arcobacters were applied to 12 batches of oysters, blue and green mussels, and paua investigated from February to May 2016. Isolates of interest were identified to genus level by 16S rRNA gene sequence comparison. Four *Arcobacter* spp. strains were recovered from three batches of green mussels. Whole-cell protein profile analysis and phenotypic characterisation delineated strains into three closely related yet distinct types. Whole genome sequence analyses revealed a markedly strong relationship among these strains, and a close genomic similarity with *A. cryaerophilus*, a species never hitherto described from marine environments. In addition, a unique plasmid containing virulence characteristics was revealed in one strain. The taxonomic position of these strains will be further explored; nonetheless we believe this to be the first description of *Arcobacter* species in New Zealand shellfish. The significance of the plasmid requires further study.

Isolation and characterisation of a new species of Campylobacter, *C. hepaticus*, responsible for Spotty Liver Disease in chickens

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The aim of this study was to identifying the agent responsible for causing Spotty Liver Disease (SLD) in Australia. SLD is a disease that most commonly occurs in egg laying hens. The disease is characterised by multiple, 1-2 mm diameter grey/white spots in the liver and a significant increase in mortality and a decrease in egg output in laying hen flocks. Bacterial isolation was attempted from the livers of SLD affected birds from farms in Australia. Homogenised liver samples were pre-enriched in modified Preston broth for two days then plated onto 5% horse blood agar and incubated at 37°C in microaerobic conditions. Representative isolates were subjected to a wide range of biochemical tests and whole genome sequencing to characterise them and identify that they represented a distinct new species of Campylobacter that we called *Campylobacter hepaticus*. The morphology of the bacteria were visualised by both scanning and transmission electron microscopy and shown to be bipolar flagellated spiral bacteria. We demonstrated that *C. hepaticus* can cause SLD be challenging healthy layer birds with in vitro grown *C. hepaticus*. 23 of 24 challenged birds developed SLD with livers lesions typical of field cases of the disease. The challenged strain could be recovered from all diseased, challenged, birds and hence Koch's postulates were fulfilled and *C. hepaticus* was definitively identified as the cause of SLD. With the etiological agent of the disease now identified further research is being undertaken to investigate disease epidemiology and develop therapeutic options to address the disease.

The immunopathogenic potential of *Arcobacter butzleri* - pathogen or commensal?

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Introduction: Given that only limited information is available about the immunopathogenic properties of *Arcobacter* infection, we here compared *A. butzleri* with intestinal pathogenic and commensal species *in vivo*.

Materials and methods: Secondary abiotic IL-10 deficient mice were generated by broad-spectrum antibiotic treatment and perorally infected with *A. butzleri, C. jejuni* or a commensal murine intestinal *E. coli* strain.

Results: Either strain stably colonized the murine intestines upon infection. At day 6 postinfection, only *C. jejuni* infected mice displayed clinical sequelae such as wasting bloody diarrhea. Gross disease was accompanied by increased numbers of colonic apoptotic cells and distinct immune cell populations. Whereas *A. butzleri* and *E. coli* infected mice were clinically unaffected, colonic immune cell numbers increased in the former, but not in the latter. Both *A. butzleri* and *C. jejuni* induced increased secretion of pro-inflammatory cytokines in large and small intestines. Remarkably, even though viable bacteria did not translocate from the intestines, systemic immune responses were induced in *C. jejuni*, but also *A. butzleri* infected mice as indicated by increased pro-inflammatory cytokine concentrations in serum samples.

Conclusion: *A. butzleri* induce less distinct pro-inflammatory sequelae as compared to *C. jejuni*, but more pronounced local and systemic immune responses than commensal *E. coli* indicating that *A. butzleri* is more than a commensal in vertebrate hosts.

Parallel session
« Genomic and Evolution »

Chairpersons:
SHEPPARD Sam, United Kingdom and DE REUSE Hilde, France

Identification of genomic changes associated with invasiveness in *Campylobacter jejuni*

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Campylobacter jejuni is the most common cause of bacterial diarrhoeal disease in the world. Clinical outcomes of infection can range from asymptomatic infection to life threatening invasive disease. This variability of outcomes for infected patients has raised questions as to whether genetic differences between *C. jejuni* isolates could lead to differences in the invasive potential of isolates, making some strains more clinically dangerous than others. In this study we compare the genomes of ten invasive *C. jejuni* isolates from Wellington, New Zealand (9 from blood samples and 1 from a joint aspirate) with reference isolates from the UK Oxfordshire surveillance project (gastrointestinal) in order to assess whether there are recurring patterns in the accumulation of mutations in protein coding genes shared by these isolates that are specific to invasive strains. Whilst there were no differences in gene content or distribution, we identified through a profile hidden Markov model technique – delta bitscore – a collection of genes that display sequence divergence patterns associated with invasive infection, including some that have been previously linked to virulence and invasiveness in *C. jejuni*. Of the top 9 genes that discriminated between the phenotype when compared to the highly curated COG database 5 of them were from the category "function unknown". *mre*B and *pgp*1 were amongst these genes, both of which are involved in the control of cell shape. However the closely related gene *pgp*2 showed no association. The same was true of other genes previously reported to be involved with an invasive phenotype (*iam*, *peb*4A, *peb*1 and *cad*F). This study presents a screen for functionally important sequence variation associated with a phenotype of interest that can be applied more broadly to other datasets, to improve our understanding of the genomic changes that occur when bacteria transition to a new niche.

Genome-wide introgression and epistasis in agricultural *Campylobacter coli*

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Campylobacter jejuni and *C. coli* are responsible for most human campylobacteriosis cases worldwide. Both species are found in a wide range of avian and mammalian hosts, most notably in farmed chicken which are the most common source of human infection through the consumption of poorly-cooked meat. It has been hypothesised that the ecological niche overlap in agricultural poultry facilitated the observed genome-wide and large-scale introgression events from *C. jejuni* to *C. coli*, whereby an average of ~10% to ~20% of the *C. coli* genome has been acquired via recombination from *C. jejuni*, as estimated by early MLST and comparative genomics work. In this study, we assembled a dataset of 827 C. coli genomes which included 689 genomes from the introgressed ST-828 and ST-1150 clonal complexes, and 138 genomes from unintrogressed clades 1, 2 and 3. Using ChromoPainter on cloud-based computer servers (MRC CLIMB), we quantified the amount and location of recombination regions in *C. coli* ST-828 and ST-1150 clonal complexes, and identified their origin from 30 *C. jejuni* clonal complexes. Introgression is expected to disrupt functional gene networks. To investigate this we a novel model of epistasis to examine the links between introgressed SNPs and those in the recipient genome. Our work addresses fundamental questions about: (i) the nature of bacterial species sharing ecological niches spatially and temporally, and (ii) the maintenance of coherent species-specific gene networks and functions. This provides improved understanding of the evolution and ecology of a major zoonotic bacterial pathogen.

Whole genome comparisons of *Campylobacter fetus* shows benefit of WGS analysis for diagnostics

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Campylobacter fetus can cause disease in humans and animals, and two subspecies are found in bovine; *Campylobacter fetus* subsp. *venerealis* (Cfv) and *C. fetus* subsp. *fetus* (Cff). Cfv is the causative agent of Bovine Genital Campylobacteriosis (BGC), a syndrome characterized by infertility and abortion in cattle, and is listed as notifiable by the OIE and is implemented for control by European legislation, whereas Cff is not. However, the current diagnostic identification assays lack sensitivity and specificity to differentiate consistently between *C. fetus* subspecies isolates

In this study we performed whole genome sequencing (WGS) on *C. fetus* strains from different hosts and with various levels of epidemiological relatedness to identify the molecular features that distinguish the subspecies. A SNP-based phylogeny was established and identified five different clades, which were not fully consistent with the biochemical characteristics of strains. Evolutionary dating identified that the Cfv clade and a Cff clade evolved recently from a Cff ancestor. The genome wide analysis showed that Cfv is most likely a Cff clone restricted to the genital tract of cattle, that relatively recently diversified from Cff.

The WGS data was used to trace subspecies specific sequences. This revealed multiple sequences, including a putative N-acetyltransferase (NAT) gene, which was used to develop a Cff and Cfv specific PCR assay, which was in full congruence with the genomic classification of strains.

Recently, a strain isolated from an artificial insemination station showed inconsistent results with biochemical and current molecular assays, and was correctly identified as Cff with the SNP quantification approach. This case showed the benefit of using WGS analysis for *C. fetus* diagnostics. WGS based diagnostics will help to establish if the current subspecies differentiation or diagnostics targeting a specific *C. fetus* clade is required for effective BGC control programs.

Recombination-driven adaptation of *Campylobacter jejuni* in cattle

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Campylobacter jejuni are globally disseminated among farmed cattle where they are thought to exist as part of the commensal gut microbiota. The emergence of livestock poultry-associated lineages has involved zoonotic transmission from other animals, potentially including wild birds but questions remain about the specific adaptations that promote proliferation in cattle and the impact this may have on transmission to humans through the food production chain. We characterised genetic variation in a population of genome-sequenced *C. jejuni* isolates of cattle origin and isolates from other hosts including poultry, wild birds, pigs and humans. Genealogical analysis identified a dominant cattle-associated sequence cluster within the ST-61 clonal complex. Using bioinformatics resources available in cloud-based computer servers as part of the MRC CLIMB project, we identified recombination events in core genes along the branch extending to the cattle-associated ST-61 complex cluster, and some accessory genes were found more often in ST-61 isolates compared to other lineages. Genomic comparison with other cattle isolates belonging to different clonal complexes may suggest homoplasy and horizontal gene transfer among cattle-associated lineages, potentially driven by cattle adaptation. By combining evolutionary analyses of *C. jejuni* genomes with predictions of putative gene function, we provide evidence of adaptation, following multiple host transitions to cattle. This has important implications for the emergence and dissemination of new pathogenic clones associated with modern agriculture.

Parallel session
«WGS and outbreak investigation»

Chairpersons:
TAUXE Robert, USA and VAN CAUTERN Dieter, France

Using genomics to investigate historic outbreaks of campylobacteriosis in the United Kingdom

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Despite the tens of thousands of cases of *Campylobacter* infection that occur in the United Kingdom each year, outbreaks of campylobacteriosis are relatively uncommon, with only four reported by Public Health England (PHE) in 2015. Since 2014, the Gastrointestinal Bacteria Reference Unit within PHE routinely adopted 7-gene multilocus sequence typing (MLST) to detect and resolve outbreaks. Prior to this, isolates were serotyped, phage typed and archived, with the evidence to resolve outbreaks provided by descriptive epidemiology.

Within its archives, PHE holds a collection of thousands of outbreak-associated *Campylobacter* isolates that were received before 2014. The aim of this study was to sequence the whole genomes of isolates belonging to multiple historic outbreaks, to determine genetic differences down to the single strain level. A variety of outbreaks were chosen for whole genome sequencing (WGS): both household and community outbreaks; those caused by contaminated poultry meat or by other sources such as water; large and small outbreaks; and outbreaks that appeared from the initial laboratory typing to be caused by either multiple strains or by a single strain.

A total of 141 isolates belonging to 14 outbreaks were chosen and recovered from frozen storage. Genomic DNA was extracted and sequenced using Illumina technology. Assembled genome sequences were examined for relatedness to a high degree of resolution using Single Nucleotide Polymorphism (SNP) typing, 53-gene ribosomal MLST (rMLST) and the 1,343-gene *Campylobacter jejuni*/*coli* core genome MLST (cgMLST) scheme available through PubMLST.

Our results suggest that WGS of outbreak isolates not only provides the high level of resolution that is needed to identify or confirm the source of the outbreak, but also allows for an insight into the natural variation that is present within the contaminating source.

A human *Campylobacter fetus* outbreak identified through next generation sequencing

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Campylobacter fetus subsp. *fetus* (*Cff*) is primarily a veterinary pathogen and occasionally associated with severe infections in humans. The high level of genome similarity in *Cff* requires high-resolution methods for studying the epidemiology. From May to October 2015 an unexpectedly high number (6) of invasive *Cff* infections in human patients were reported in the province of Zeeland (n=5) and the neighbouring province of Brabant (n=1) in the Netherlands. Based on epidemiological information and patient questionnaires, it was concluded that the patient from Brabant most probably acquired the infection abroad, whereas all patients in Zeeland consumed unripened sheep cheese from unpasteurized milk. For 4 patients, the product could be traced to one sheep farm, while for the 5th patient a second farm was indicated.
Microbiological investigation of sheep faeces revealed *Cff* in sheep from the first farm but not of the second. MiSeq genome sequencing was used to investigate isolates from patients, from the suspected sheep flock, and a set of Dutch *Cff* reference genomes from human and ruminant origin. The Harvest suite was used for core-genome alignment and reconstruction of single-nucleotide polymorphism (SNP) based phylogeny.
All isolates belonged to MLST ST6, and SNP phylogeny irrevocably showed that 5 isolates belonged to a distinct clone while the 6th isolate from the unrelated patient belonged to a separate clone. The core genome of 5 isolates generally differed between 0-7 SNPs, while epidemiological unrelated human isolates differed by at least 26 SNPs. The outbreak isolates harboured determinants for serum resistance (*glf*) and the surface array protein (*sap*), both associated with invasive infections. The genomes from the sheep isolates were identical to the patient isolates, confirming the epidemiological source identification. Conclusions: NGS confirmed that faecal contamination of sheep milk and consumption of unpasteurized cheese lead to an outbreak of *Cff* infections.

Whole-genome sequencing of *Campylobacter jejuni* isolates from patients reveals numerous genetic clusters and possible epidemiologically linked cases

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Introduction: It is widely believed that most *Campylobacter jejuni* infections are sporadic. However, the frequency of *C. jejuni* outbreaks is likely to be underestimated as high-discriminatory typing methods are rarely used for detection of *Campylobacter* outbreaks. Whole-genome sequencing (WGS) has the potential to give new insights in the epidemiology of campylobacteriosis, e.g. to determine the proportion of mini-clusters and outbreaks, which may previously have gone undetected. Here, we present the results of WGS performed on a random selection of *C. jejuni* isolates submitted to Statens Serum Institut during a 9-month period in 2015-2016, covering four geographically dispersed and representative areas of Denmark.

Material and methods: 245 *C. jejuni* isolates were sequenced, assembled, and MLST were extracted. SNP analysis was performed on isolates belonging to the same ST. Genetic clusters were defined when two or more genomes clustered together and clearly separated from other genomes within the same ST.

Results: A total of 78 sequence types (ST) were identified of which 6 included more than 10 isolates and 44 STs only one isolate. 62 isolates made up 23 genetic clusters, each involving 2-8 patients. Thus, 25% of this random selection of isolates was part of a genetic cluster. One cluster involving 8 persons was restricted to a small geographical area within a few weeks, and by interviewing patients it was found likely that they were infected by a specific batch of green salad. Other genetic clusters were also restricted in space and/or time.

Conclusion: WGS-analysis of isolates from only a fraction of the total number of cases demonstrated that *C. jejuni* case clustering, and even outbreaks, occur with a higher frequency than previously assumed. The results show the potential of future large-scale application of WGS for *C. jejuni* outbreak detection, confident case definition and the possibility of linking to food sources.

Swedish winter peaks of Campylobacter in humans and chicken are connected

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Campylobacter is the most reported zoonotic bacterial cause of gastroenteritis in Europe. In Sweden, it is compulsory to report all laboratory-confirmed cases of campylobacteriosis. Within the Campylobacter broiler monitoring programme, all flocks are sampled and analysed for the presence of Campylobacter. Most cases of campylobacteriosis and the highest prevalence of Campylobacter in broilers in Sweden are reported during the summer months, but additional peaks were observed in the early winters of 2014 and 2015. The aim of this study was to analyse if the temporary increases of domestic cases of campylobacteriosis in the winters of 2014 and 2015 were linked to the increase of Campylobacter in broilers during the same time periods.

Isolates from humans and broilers were randomly selected, but represented a broad geographical origin. Isolates from the first winter peak were analysed by pulsed-field gel electrophoresis prior to whole genome sequencing (WGS), but isolates from the second peak were directly subjected to WGS. The phylogenetic relationship between the genomes were analysed with SNP-analysis. The majority of the isolates clustered within a few clusters containing both human and broiler isolates. These clusters corresponded to individual ST-types: ST-48, ST-21 and ST-50 (winter 2014) and ST-48 and ST-257 (winter 2015). One cluster (ST-1326) contained only broiler isolates. Isolates of ST-257, which has previously been connected with campylobacteriosis in Sweden, were compared to isolates collected from earlier years. The results indicate that one strain of ST-257 is a recurrent cause of campylobacteriosis in Sweden.

This study showed that the peaks of domestic cases of campylobacteriosis in the winters of 2014 and 2015 were clearly linked to the increases of Campylobacter in broilers.

Ad hoc whole-genome MLST (wgMLST) analysis reveals multiple _Campylobacter jejuni_ clones caused a massive waterborne outbreak in New Zealand in 2016

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Bacterial infection outbreaks are usually thought to be caused by genetically identical isolates (clones). However, in the investigation of a recent *C. jejuni* outbreak in Havelock North, New Zealand, whole-genome based analysis revealed a different pattern. In August 2016 over one third of the 14,000 local residents were sickened with vomiting and diarrhea. Later *C. jejuni* was isolated from the reticulated water. Over 80 *C. jejuni* isolates from patients and environmental samples were studied with whole-genome sequencing (WGS) and *ad hoc* wgMLST analysis. The majority of the isolates belonged to sequence type (ST) 42 and 3610. Epidemiologically linked human isolates were also found belonging to ST1517, ST50 and ST474. Some of the isolates were found to be carrying a large unreported genomic island. Using *ad hoc* wgMLST and subsequent phylogenetic analysis we found evidence of clonal expansion in certain branches of both ST42 and ST3610. These branches contained patient isolates mixed with water isolates and ovine isolates from fields adjacent to the wells supplying the reticulated water, suggesting their close relationship and a likely origin of contamination of the water supply. Moreover, as the *ad hoc* wgMLST identified more allelic differences comparing to the wgMLST analysis with a fixed scheme, the hidden relatedness of multiple clones of the same ST to the outbreak was revealed. Some of the human isolates found in this investigation did not fall into any branches that had undergone clonal expansion, also supporting the hypothesis that the outbreak was caused by multiple clones of *C. jejuni*. The composition of the isolates in our study likely a reflection of the composition of the population structure in the original source. Our finding confirmed the value of WGS-based analysis in drawing the ultra-fine map of the relationships in closely related isolates, as well as the value in epidemiological investigation.

Comparing the genomic epidemiology of two 'slow-burn' epidemics of *Campylobacter jejuni* in New Zealand

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New Zealand has experienced relatively high rates of poultry-associated campylobacteriosis over the last two decades. Although human cases have been associated with many diverse multilocus sequence types, there have been some notable, dominant sequence types (STs) that have emerged and caused widespread human infection over multiple years. In this study we compare the genomic epidemiology of two such STs; one associated with a single poultry supply (ST474, belonging to clonal complex 48) and the other a more recently emerged fluoroquinolone and tetracycline resistant *C. jejuni* (ST6964, belonging to clonal complex 354) that spread rapidly among all major poultry supplies in the North Island, following its emergence in 2014. Using Illumina and Pacbio whole genome sequencing (WGS) of 402 ST474 and 230 ST6964 isolates, collected between 2005 and 2016 from humans, poultry, ruminants, wildlife and the environment, we describe how these STs evolved through the acquisition of point mutations, phage insertions, plasmids and natural transformation. We provide evidence of the existence of a 'basal clade' of ST474, possibly associated with ruminants, from which emerged a poultry-associated clade that accounted for a large increase in human cases up to 2006/7. Following interventions in the poultry industry in 2007/8, the number of notified human cases declined by 50% and this 'epidemic clade' was replaced by a distinct new clade. This clade gradually declined in prevalence over the subsequent 4 years, with most recent isolates reverting to the 'basal clade'. In 2014 ST6964 emerged to become the most prevalent ST in all poultry sources and a common cause of human campylobacteriosis. However, the emergence of this ST was not associated with an increase in human cases. WGS combined with evolutionary modelling has provided unprecedented insight into the rapid evolution and transmission of *C. jejuni* of considerable public health importance in New Zealand.

Parallel session
«Tutti Campy»

Chairpersons:
HADDAD Nabila, France and FEDERIGHI Michel, France

Caecal microbiome modifications across age in a free-range poultry flock naturally-infected with *Campylobacter*

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A free-range poultry flock reared for 82 days was monitored for bacterial community composition and *Campylobacter* infection by collecting samples at 4, 18, 39, 58 and 81 days-of-age. Cloacal swabs were analysed for the presence of *Campylobacter* by culture and real-time PCR; selected colonies were characterised by *fla*A PCR-RFLP and MLST. Caecal DNA samples were subjected to Illumina MiSeq paired-end sequencing of 16S rRNA amplicons (V4 region). *Campylobacter* was first detected when birds were 39 days-old; *C. coli* was isolated and both *C. coli* and *C. jejuni* were detected by PCR. Animals remained infected throughout their productive life and both species were isolated during the last two samplings. At least two different *C. jejuni* strains and 4 *C. coli* were identified. 16S amplicon-based high-throughput sequencing identified *Campylobacter* as one of the differentially abundant taxa across age. In agreement with microbiology, no *Campylobacter* sequences were found until day 39 when relative abundance was highest and started to decrease significantly afterwards ($p<0.001$). Several taxa were positively correlated with *Campylobacter* abundance (Spearman's rank correlation), including genera *Odoribacter*, *Helicobacter* and *Megamonas*, and families *Rikenellaceae* and *Lachnospiraceae*. Age was associated with significant differences in alpha and beta diversity indices of bacterial community composition. Firmicutes and Proteobacteria were the only phyla identified in 4-day-old chickens, their abundance decreased in older chickens as Bacteroidetes became dominant (detected from day 39 onwards). *Enterobacteriaceae* (phylum Proteobacteria) reached highest levels at day 4 and sharply declined afterwards. Within Firmicutes, *Lachnospiraceae* outnumbered *Ruminococcaceae* at age 4. This dominance switched at age 18 but their levels became even as *Rikenellaceae* and *Bacteroidaceae* (phylum Bacteroidetes) gained importance. Relative abundance of other zoonotic bacteria, like *Helicobacter pullorum* and *Clostridium difficile* also differed significantly among age-groups. Understanding diversity and shifts in chicken caecal microbiota composition could help to design strategies to control zoonotic infections in animals.

Comparing *Campylobacter jejuni* isolated from organically and conventionally raised chickens in Québec by whole genome sequencing: nothing special to be signalled.

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The poultry industry is pressured to reduce non-therapeutic antibiotic usage. Organic chicken production does not use antibiotics and therefore its study may predict the effects of reducing antibiotics in conventional farms. *Campylobacter jejuni* colonizes chickens raised according to both production types. The objective of this study was to compare the genomes of *C. jejuni* strains originating from organic (n=12) and conventional (n=16) productions to identify traits associated with organic chicken. *C. jejuni* strains were sequenced on an Illumina MiSeq. Genomes were assembled using INNUca and annotated by PROKKA. Pan-genome comparison was achieved using Roary and Scoary. Core-genome analysis was performed with Parsnp firstly on the strains from this study, and again following the inclusion of 800 multi-host genomes downloaded from PATRIC. The presence of antimicrobial resistance genes was assessed using CARD. Analyzed organic strains belonged to ST 8, 45, 267 and 1 212 with only ST 1 212 being absent from the conventional strains. Scoary returned 96 genes that associated with production type but none had a Bonferonni adjusted *p* value less than 0.05. Four genes had a naïve *p* value lower than 0.01 and only *por*A was associated with organic production. Trees generated by Parsnp showed no grouping of strains by their production origin. As for antimicrobial genes, for both production categories, half of all strains harboured the tetracycline resistance gene *tet*O. Aminoglycoside resistance gene *APH* was found in 3 conventional strains. Genes *OXA*-61 and *OXA*-184, coding for beta-lactam resistance, were found in conventional strains while *OXA*-61 was identified in all organic strains. These results suggest that strains found in organic chickens do not have significant genomic differences from strains originating from conventional poultry production systems.

Transcriptomic and functional evaluation of the small RNA CjNC110 in *Campylobacter jejuni*

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Recent advances in the use of high throughput deep sequencing of RNA (RNAseq) have revolutionized the study of gene expression and identification of small non-coding RNAs in pathogenic microorganisms. While multiple studies over the past several years have identified the presence of non-coding RNAs in the transcriptomes of various strains of the zoonotic pathogen *Campylobacter jejuni*, few have attempted to elucidate the functional role of these newly identified regulatory genes. Due to its intergenic location immediately downstream of the *luxS* gene, the small RNA CjNC110 was chosen by our lab for further study. Deletional mutagenesis was utilized to create a knockout mutant of CjNC110 as well as a double knockout of *luxS* and CjNC110 in the IA3902 isolate of *C. jejuni* sheep abortion (SA) clone. Strand specific high throughput RNA sequencing of stationary and exponential growth phases was then completed and Rockhopper used to generate a list of potential mRNA regulatory targets of CjNC110. Results of the RNAseq demonstrated differential expression of a number of genes involved in important metabolic pathways such as energy taxis, flagellar glycosylation, and quorum sensing with the most significant changes observed in the *luxS*/CjNC110 double knockout mutant. Phenotypic assessment of these pathways using growth in defined media, motility in semi-solid agar, autoagglutination ability, and autoinducer-2 (AI-2) production also revealed statistically significant differences between wild type and mutant strains. Complementation of the CjNC110 mutation under a constitutive promoter led to over-correction of many of the differences observed in the phenotypic assays, suggesting that titration of the amount of CjNC110 present in the cellular environment may allow for regulation of gene expression within these important metabolic pathways.

The effect of the timing of exposure to *Campylobacter jejuni* on the microbiome and inflammatory responses of broiler chickens

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Poultry are a major source of *Campylobacter* foodborne disease. The objective of this study was to compare the changes in microbiota, inflammatory responses and zootechnical parameters that occur when campylobacters are introduced to broiler chickens at an early stage in development at 6 d, with those at 20 d old when campylobacters are frequently detected in commercial flocks. The data collected included: viable counts of *C. jejuni* in caecal contents; microbiome analysis; cytokine gene expression analysis; histological investigation and bird weight and feed consumption data. Birds infected with *Campylobacter* at 20 days became colonized within 2 d of exposure, whereas birds infected at 6 d of age did not show complete colonization until 9 d post-infection. The caecal microbiota of birds infected with *Campylobacter* were significantly different to age-matched uninfected controls at 2 d post-infection, but generally the age of the birds had a greater effect on diversity than introduction of *Campylobacter*. Reductions in the relative abundance of OTUs within the taxonomic families *Lactobacillaceae*, *Coriobacteriaceae* and *Lachnospiraceae* were observed. Analysis of caecal chemokine/cytokine gene expression revealed increases in IL-6, IL-17A and Il-17F consistent with a Th17 response but the persistence of the response was dependent on the age of *C. jejuni* colonisation that coincided with caecal IL-10 responses. No difference in bird performance was associated with *Campylobacter* infection at either age, and although some histological changes were observed, the effect was transient. In conclusion, it is evident that a sudden shift in microbiota, caused by the introduction and colonization of a highly successful enteric bacterium at either 6 d, or at 20 d, elicits a pro-inflammatory response and minor observable histological changes but the outcome is to establish tolerance.

The river runs through: Understanding the human health risks of *Campylobacter jejuni* and *Campylobacter coli* in recreational waters.

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Campylobacter is a global leading cause of bacterial gastroenteritis. Water represents an environmental sink, and reservoir, for this organism. However, not all species and sub-types, present within this environment, are associated with human disease; leading to inaccuracies in quantitative microbial risk assessments (QMRA).

Multi-Locus Sequence Typing (MLST) has been applied to evaluate the pathogenic risk presented by campylobacters. However, whole genome sequencing (WGS) now offers an alternative risk assessment method. Evaluations can now be based on, not only, MLST types, but also, the presence/absence of virulence and antibiotic resistance genes.

The Yarra River (Victoria; Australia) supports numerous, economic, environmental and social values. Results of previous QMRAs identified *Campylobacter* as an organism of concern due to its consistently high concentration within the river. Thus, to understand and define the risk presented by *Campylobacter* a WGS-MLST study was undertaken on 72 isolates from riverine, stormwater and estuarine sources. Source attribution was conducted and the relative human health risks were defined through comparison to previously typed isolates (from global and regionally-specific sources), relevant clinical isolates and investigation of antibiotic resistance and virulence gene carriage.

Taxonomic classification demonstrated that *C. coli* was the most abundant species (82% vs. 18% *C. jejuni*). A total of 43 unique sequence types (ST) were defined; of these 38 represented new STs. *C. jejuni* isolates were primarily attributed to dogs (4% *C. jejuni*; P = 0.02) and humans (0.9%; P = 0.005). In contrast, *C. coli* was associated with cattle (32% *C. coli*; P = 0.02) and ducks (12.3%; P = 0.004). Antibiotic resistance cassettes and key virulence factors were identified within at least 10% of water isolates. These data were applied to generate a risk estimate; the findings of which suggested that ~26% of waterborne Yarra River *Campylobacter* isolates represent a human disease risk.

Developing a comparative conditional incidence to analyze relationships between local weather and Campylobacter infections in England and Wales

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Seasonality in Campylobacteriosis is poorly understood, with data exhibiting puzzling patterns such as a steep increase in the incidence in England and Wales during the early summer. Environmental factors are expected to play an important role, but disentangling and quantifying the contribution of each driver in Campylobacteriosis is problematic as the time-varying explanatory variables are often related (collinearity).

We linked a dataset of 1.2 million Campylobacter cases over 25 years in England and Wales with meteorological datasets from the national weather service at diagnostic laboratory location. Data were extracted for the date when specimen reached the laboratory and previous 90 days. The chosen variables were minimum and maximum temperature, relative-humidity, rainfall and daylight duration.

We analyzed the subsets of cases when the explanatory variables, except one, were within the same narrow range (e.g., all cases with averaged rainfall and relative-humidity between 5-10 mm and 70-75%, respectively). This allowed us to: i) detect the explanatory variables and remove collinearity; and ii) quantify the probability of acquiring the disease conditional on the weather variables averaged for a given time-lag.

The method was validated by reconstructing the time-series of events as Poisson processes. This method accurately reproduced the empirical patterns when the variables were averaged over the previous two months.

We found that rainfall is not an explanatory variable per se. In contrast, the steep increase in incidence in early summer and inter-annual variations are associated with temperature, relative humidity and daylight duration only. The risk of infection increased non-linearly with two-months averaged prior maximum temperature; and varies non-monotonically with two-months averaged prior relative humidity. In particular the risk is highest for relative humidity between 75-80% and maximum temperature 14-16 °C.

We have developed a robust, conceptually simple, method to identify the relevant weather variables involved in Campylobacteriosis, and disentangled their relative contribution.

Plenary session
«Epidemiology and Public Health »

Chairpersons:
TAUXE Robert, USA and VAN CAUTERN Dieter, France

Significant Implication of Ruminants in Human Campylobacteriosis in France: Three Genotyping Methods for a Same Trend in Source Attribution?

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Campylobacter is responsible for the most frequently bacterial foodborne gastroenteritis in Europe. In recent years, many genotyping methods were developed to describe *Campylobacter* populations enhancing the understanding of their epidemiology and their genetic structure. However, despite the significant availability of genotyping data, the transmission routes and the relative contribution of the different reservoirs to human infection remain unclear. In this study, we assessed the implication of chicken, ruminants, pets and environmental waters in human campylobacteriosis in France and compared the ability of three genotyping methods (CGF40, MLST and WGS) to identify the origin of clinical cases. A collection of 2132 *C. jejuni* isolated from various reservoirs and clinical cases were characterized using the CGF40 method. A subset of 1067 *C. jejuni* was selected for characterization using MLST and a selection of 370 isolates was whole genome sequenced. Using the molecular genotyping data obtained with each method, source attribution was performed to identify the origin of human campylobacteriosis in France. To improve the source attribution based on WGS data, 15 epidemiological markers were identified as host-segregating markers and used to attribute an origin to human campylobacteriosis. Therefore, when applying this approach on WGS data, it appeared that 45.8% of clinical cases were attributed to chicken and 46.9% to ruminant reservoirs while a small part of cases was attributed to the environment and pets. Consistent results of source attribution were also observed using MLST and CGF40 genotyping data. These results highlighted the congruence between the genotyping methods for source attribution and constituted the first data on source attribution in *C. jejuni* in France. It emphasized the importance of chicken as a main source of campylobacteriosis, and interestingly revealed the ruminant reservoir as a significant transmission route of *C. jejuni* to human in France.

How results of campylobacteriosis case-control/case-case studies vary depending on their design?

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Introduction: In Canada, campylobacteriosis and other enteric diseases are reportable. The systematic collection of epidemiological data from such cases allows for case-case comparisons on potential risk factors in order to inform prevention and control. A survey of healthy people on their exposures to known risk factors for enteric pathogens was carried out over one year in Ontario. Data from this survey and from enhanced surveillance in the same region were used to perform a case-control study for campylobacteriosis since this design should provide more valid results than case-case comparisons. Considering the extra cost of having controls for case-control analysis, the present study aimed at empirically answering whether the differences in results between case-control and case-case designs are so large that case-case designs cannot replace the more expensive case-control design.

Methods: We defined 15 scenarios of mixed designs (individually-, population-matched or unmatched case-control, and case-case) with various periods of data for the cases (2, 4 or 6 years). For each scenario, we built a logistic regression model for 35 individual potential risk factors adjusting for age and season. We tested the homogeneity of odd ratios between scenarios using the I2 statistics. We assessed similarity between scenarios through a hierarchical agglomerative clustering analysis based on the distance between the beta coefficients of the logistic regression.

Results: There was an overall disagreement between all scenarios for each potential risk factor tested but one. The clustering analysis showed two main groups of scenarios, one including the 9 case-case designs and the other 6 case-control designs. The number of years of data had limited impact.

Conclusion: The overall results of case-control/case-case analysis for campylobacteriosis vary significantly with the study design. The true case-control study is worthwhile. For some specific risk factors, the impact of design is minimal and the cases-case design may well be acceptable.

Infection pressure by *Campylobacter* and associated risk factors: a seroepidemiological study in the Netherlands

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Background: Among *Campylobacter* infections, those symptomatic represent only the tip of the iceberg and may show a different picture of risk factors, limiting our understanding of *Campylobacter* epidemiology. This population-based study presents an estimation of *Campylobacter* infection pressure and associated risk factors.

Methods: A two-stage population-based serological survey was performed in the Netherlands between February 2006 and June 2007. Levels of IgG, IgM and IgA against *Campylobacter* were determined using ELISA and used to estimate incidence rates (as a proxy for infection pressure) based on reference peak levels and decay rates over time of these parameters after infection by *Campylobacter* using the ECDC serocalculator. Multivariable generalized linear models were used to identify risk factors for infection. Sampling weights were applied for estimating overall infection pressure.

Results: We analyzed sera from 1,559 patients: 43.9% were males, 28.9% under 15 years of age, 15.7% of non-Dutch ascent, and 12.4% living in rural settings. Estimated overall infection pressure was 1.61 infections per person-year (95%CI: 1.58-1.64). Factors independently associated with increased infection pressure were: increasing age [vs. 15-34 years, for <5, 5-14, 35-54 and 55-70: exp(β)=0.60(0.58-0.63), 0.74(0.71-0.78), 1.08(1.03-1.13) and 1.08(1.01-1.16), respectively; all p<0.01], non-Western ethnicity [vs. Dutch, Moroccan/Turkish, Caribbean and other non-Western ethnicity: exp(β)=1.25(1.14-1.37), 1.14(1.03-1.25) and 1.09(1.02-1.17), respectively; all p<0.05], female gender [exp(β)=1.07(1.04-1.11); p<0.01], having travelled abroad [exp(β)=1.05(1.01-1.09); p=0.01], having eaten half-cooked meat in the last year [exp(β)=1.04(1.01-1.08); p=0.02], and living in a lower socioeconomic status (SES) area [vs. higher exp(β)=1.05(1.01-1.10); p=0.01].

Conclusions: Infection pressure by *Campylobacter* is much higher than incidence of symptomatic cases (5.6/1000 person-years). Demographic factors (age, gender, ethnicity and SES) probably act as proxies for hitherto unmeasured exposures. Surprisingly, contact with animals and rural setting did not appear significant, while travelling abroad and consuming undercooked meat had small but significant effects.

Changing diagnostic testing practices and impact on incidence of *Campylobacter* infection– Foodborne Diseases Active Surveillance Network, USA, 2012-2016

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Introduction: *Campylobacter* is estimated to be the most common bacterial enteric infection in the United States. Use of culture-independent diagnostic tests (CIDT) to diagnose enteric infections is increasing.

Methods: The Foodborne Diseases Active Surveillance Network (FoodNet) conducts surveillance in ten sites for *Campylobacter* infections and surveys of clinical laboratories to understand diagnostic testing practices.

Results: Among 39,979 *Campylobacter* infections reported during 2012–2016, 31,888 (80%) were diagnosed by culture and 8,091 (20%) by CIDT. CIDT reports increased from 12% of cases in 2012 to 32% in 2016. Inclusion of CIDTs in *Campylobacter* case counts increases the incidence rate (IR) by 13% in 2012 (16.1 [with CIDT] vs 14.2 [without CIDT] per 100,000) and 47% in 2016 (17.4 vs 11.8). Among 7,041 CIDT reports with test type, 69% were antigen-based, 30% PCR-based, and <1% PCR-based syndrome panel tests. The proportion of cases diagnosed with any PCR-based test increased from 9% in 2012 to 45% in 2016. Compared with patients diagnosed by antigen-based tests, those diagnosed by PCR-based tests were younger (34 vs 47 years), less likely to be female, white, or hospitalized, and more likely to be Hispanic, have diarrhea, fever, or an international travel history in the seven days before illness. Among laboratories using CIDTs in 2016, 64% performed or sent a specimen out for reflex culture.

Conclusion: The proportion of *Campylobacter* infections diagnosed by CIDTs has increased and there has been a shift in the type of CIDT used. Differences among persons diagnosed by PCR-based compared to antigen-based tests may reflect differences in community diagnostic testing practices. Reflex culture is not universally done, restricting determination of species, subtype, and antimicrobial susceptibility. More work is needed to understand how best to incorporate CIDT into *Campylobacter* surveillance.

Risk factors for sporadic *Campylobacter* infections in Denmark: a case-control study in children and young adults

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Introduction: *Campylobacter* is the most prevalent cause of bacterial gastroenteritis in Denmark with 4,676 cases (81/100.000 persons) reported in 2016. Infection is linked to foreign travel and consumption of poultry, however many domestically acquired cases appear related to non-food risk factors, the identification of which through case-control studies is hampered by acquired immunity in older age groups. We report a study focusing on identifying non-food related risk factors for domestic cases.

Materials & Methods: During 2016, all patients living in Denmark aged <31 years with confirmed *Campylobacter* infection were invited to complete an on-line questionnaire on factors such as travel, food, animal contact and recreational activities. Separate questionnaires were used for children and adults. Controls aged <31 years were randomly selected from the Danish population register. Uni- and multivariable analyses were adjusted for gender and age.

Results: A total of 1 366 cases and 4 418 controls were included in the study, response rate 67% and 66%, respectively. Domestic campylobacteriosis was associated with consumption of different chicken cuts and barbecued or microwaved meats. Further risk factors were: fresh water swimming, drinking water from a stream or well, contact to animal faeces from dogs with diarrhoea. For children specifically, cases more often swam in the ocean, played in the sand, ate outdoor meals/picnicked and drank unpasteurized milk. Overall, multivariable analyses identified the same set of risk factors. For persons who reported travelling before symptom onset, risk of disease was significantly associated with travel to Thailand, Indonesia, Turkey and Africa, eating food from street kitchens and having contact to sand and water during their travels.

Conclusions: This large case-control study in a population of young individuals confirms foreign travel and chicken consumption as risk factors. Further, it identifies environmental and recreational risk factors such as contact to water, sand and animal faeces and behavioral risk factors specific for children. As a unique feature, this study also identified risk factors for persons traveling abroad. All of these findings have potentially important preventive value.

A combined case-control and molecular source attribution study of human *Campylobacter* infections in Germany, 2011-2014

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Campylobacter infection is the most commonly notified bacterial enteritis in Germany and in many other countries. We performed a large case-control study to identify risk factors for sporadic *Campylobacter* infections in Germany and combined it with a source attribution study to determine the relative importance of various animal sources for human infections. Case patients and randomly selected controls completed a questionnaire (study period Nov 2011-Feb 2014). We conducted multivariable logistic regression analysis to identify risk factors. Source attribution analysis was performed using the asymmetric island model (AIM) based on MLST data of human and animal/food isolates. As animal sources we considered chicken, pig, pet dog or cat, cattle, and poultry other than chicken. We analysed questionnaires from 1,812 case patients with autochthonous *Campylobacter* infections and 3,983 control persons. Consumption of chicken meat (adjusted odds ratio (aOR): 1.6; 95% confidence interval (CI) 1.2-2.0; population attributable fraction (PAF) 31%) and eating out (aOR 1.6; 95% CI 1.3-2.0; PAF 30%) were the most important risk factors for *Campylobacter* infections. Additional risk factors were preparation of poultry meat in the household (aOR 1.4; 95% CI 1.1-1.8; PAF 14%); preparation of uncooked food and raw meat in the household at the same time (aOR 1.3; 1.1-1.5; PAF 12%); contact with poultry animals (aOR 2.1; 95% CI 1.4-3.0; PAF 3%); and the use of gastric acid inhibitors (aOR 1.9; 95% CI 1.5-2.3; PAF 10%). A total of 613 human isolates were analysed by AIM. The mean probability of human *C. jejuni* isolates to originate from chickens was highest (74%), whereas pigs were a negligible source for human *C. jejuni* infections. Human *C. coli* isolates were likely to originate from chickens (56%) or from pigs (32%). Efforts need to be intensified along the food chain to reduce *Campylobacter* load, especially on chicken meat.

Poster session
« Genomic and Evolution »

How open is the Campylobacter genome?

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Many comparative genomics studies exploit the concepts of the core- and pan- genomes to examine similarities and differences between different strains. On the other hand, phylogenetic analyses seek to examine the evolutionary origins of different lineages from non-mobile, core genetic elements. But even in closely-related lineages, random mutations that result in quantifiable tree-like phylogenetic ancestries are only a single class of the genetic strategies used to adapt to changing environments.

One of the main goals for genomic data in epidemiology is to determine how different classes of evolutionary change have undergone selection to result in the acquisition of traits, such as virulence. It is, thus, important to develop new strategies to understand these evolutionary changes particularly as sequencing costs decrease and comparative genomics analyses increase in their complexity. Going beyond the analysis of core genes, and the limitations of phylogenetics is particularly relevant for prokaryotic organisms such as Campylobacter and Helicobacter, organisms with high rates of recombination and horizontal gene transfer.

Here, we explore the dynamic composition of Campylobacter core- and pan- genomes within whole-genome derived phylogenies. Using real-world epidemiological data acquired over ten years of Campylobacter surveillance in New Zealand, we estimate the evolutionary time-scale over which “openness” is deemed to be an important driver of Campylobacter evolution. Further, we re-examine the use of Heap’s Law for defining the composition of the pangenome within structured ancestries and go on to propose a simplistic strategy for integrating phylogenetic analyses with genomic data.

We conclude that many of the complex evolutionary behaviours of Campylobacter cannot be generalised and are often context dependent. Thus understanding their evolution, or indeed that of any prokaryotic organism, requires a holistic approach to maximise the utility of the abundance of data generated from genomics.

The genetic diversity and evolution of plasmids from *Campylobacter jejuni* and *Campylobacter coli* from the United Kingdom (UK)

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Introduction: Bacterial plasmids facilitate the dissemination of virulence and antibiotic resistance genes; however, relatively few *Campylobacter* plasmids have been characterised to date. In this study, we leveraged whole-genome sequence (WGS) data to investigate the genetic diversity and evolution of plasmids from *Campylobacter jejuni* and *Campylobacter coli*.

Methods: A pilot study was carried out on 494 human disease isolates collected in Oxfordshire, UK (2014-2015). Short-read data were assembled with plasmidSPAdes, an algorithm for assembling plasmids from WGS data. The resulting assemblies were queried against the NCBI nucleotide database; putative plasmids were annotated using a combination of Prokka and Bacterial Isolate Sequence database (BIGSdb) annotation tools in the PubMLST database. Gene-by-gene comparisons of plasmids were carried out using the BIGSdb Genome Comparator module. The *C. jejuni/coli* PubMLST database (https://pubmlst.org/campylobacter/) contained whole-genome assemblies for all study isolates, which facilitated genomic comparisons of those carrying plasmids.

Results: Of the 408 assemblies generated by plasmidSPAdes, 145 (35.5%) were identified as putative plasmids. Plasmids were found in 134/494 (27.1%) study isolates, 123 (91.8%) of which carried one plasmid each. The putative plasmids varied in size (1.3 kb to >70 kb), with GC content ranging from 20-39%. The majority of plasmids (~66.4%) were homologous to pTet, a tetracycline resistance plasmid; however, these sequences were variable, particularly with respect to the 'cargo' region, which carries antibiotic resistance and virulence determinants. Most (26.7%) of the remaining plasmids were homologous to pCC42yr, a conjugative cryptic plasmid. The predominant plasmids were found in both *C. jejuni* and *C. coli* isolates, which were genetically diverse at the genome level, encompassing several clonal complexes and characteristic of diverse host sources.

Conclusions: This study has provided an overview of plasmids in *C. jejuni* and *C. coli*. Further analyses will provide additional insights into the distribution, diversity, and evolution of plasmids in *Campylobacter*.

Campylobacter Strain Diversity – *Campylobacter* is dead; long live *Campylobacter*

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One of the characteristic features of *Campylobacter* is the very large number of strains that are found across its diverse array of host species. This has led to challenges in identifying the main sources of human infection however neutral genomic markers in combination with molecular attribution methods are now clarifying this.

In Scotland, and more specifically in Grampian region, *Campylobacter* isolates have been collected and genotyped from both human cases and from all of the major animal and bird reservoirs since 2000. As of 2017 this collection now comprises some 9,750 clinical isolates, with typically some 500 cases each year, and also isolates from retail chicken (1,250), cattle (850), sheep (650), pig (150), and wild birds and other sources (200). These have all been typed by 7-10 locus MLST and since 2011 with whole genome sequencing.

Strain turnover over this 17 years period has been examined using a combination of graphical visualisation and of estimates of the date of strain origins. It is apparent that underlying this population diversity there are contributions from strains which have existed for a long period of time whilst there are other strains that are newly arisen and others that have faded in relative abundance across these sources.

The implication of strain turnover for studies on evolution and of source attribution studies and of how these relate to the reservoirs and, particularly to broiler production are discussed.

Diversity among UK *Campylobacter jejuni* NCTC 11168 laboratory strains

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Campylobacter jejuni NCTC 11168 is the most commonly used laboratory reference strain worldwide, and has greatly improved understanding of this organism. It is widely known among the *Campylobacter* research community that there are phenotypic variations in this strain, with the most reported difference being in motility, with a motile and non-motile variant having been identified. We collected 26 *Campylobacter* 11168 isolates from laboratories across the UK and sequenced their genomes to investigate genetic variations that might underlie phenotypic differences. In addition to whole genome sequencing, phenotypic testing including growth at 37 and 42^{o}C, motility, invasion assay into human and avian cell lines and resistance to Ampicillin were also tested. Differences in motility among strains were confirmed and variation in growth rate at both 37 and 42^{o}C was also observed. The strains were capable of invasion of both human and avian cell lines and there were differences between strains, with the less motile ones having poorer invasion. Comparative genomic analysis revealed the accumulation of synonymous and non-synonymous sequence variation within the core genome of different strains that was related to the history of transfer of the progenitor strain (Public Health England) between UK laboratories. Accessory genome variation was also observed with some strains containing functional bla-Ox genes, confirmed with phenotypic testing. This study provides evidence of ongoing evolutionary change among isolates that are usually considered isogenic and highlights the need for careful consideration of genetic variation within laboratory reference strains.

Genetic diversity of *Campylobacter* in wild birds

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Campylobacteriosis is the most prevalent bacterial zoonotic disease worldwide. Wild birds can act as reservoirs of *Campylobacter* representing a risk to human and animal health.
We investigated the carriage status and the pathogenic potential of *Campylobacter* isolated from wild birds in Italy. A total of 27 strains isolated during a passive surveillance program were identified with PCR methods and whole-genome sequencing (WGS). Genomes were sequenced with Illumina technology and assembled *de novo* with SPAdes 3.8.1. Genome assemblies quality was improved using Pilon 1.8. MLST and cgMLST typing were performed using Ridom 3.2.1. *C. jejuni* was isolated in 12 pigeons (*Columba livia*), 5 magpie (*Pica pica*), 3 crows (*Corvus Frugilegus*), 2 greenfinch (*Chloris chloris*), 1 whitewagtail (*Motacilla alba*), 1 owl (*Asio otus*), 1 starling (*Sturnus vulgaris*), *C. coli* was isolated in 1 great crested grebe (*Podiceps cristatus*), while one strain from a heron (*Ardea cinerea*) was not assigned to a specific *Campylobacter* spp. The sequence of the 16s rDNA gene resulted to the potentially identification of *C. volucris*. The genome assembly from the heron isolate confirmed the *C. volucris* species by the Genome-to-Genome Distance Calculator (GGDC), an in silico DNA-DNA hybridization method (DDH), using the DDH model "formula 2" as recommended for draft genomes. The MLST revealed 6 different CCs and 14 STs with seven of them never recorded before. The most frequent CCs were the "generalist" ST45 (29.62%) and ST179 (11.11%), confirming their association to the wild birds reservoir. The ST403, notoriously reported in human disease was also found. *C. volucris* putative coding sequences (CDSs) were determined using Prokka and a comparison with *C. volucris* LMC 24379 revealed a difference of gene identity>11% across the query and match sequence. These findings confirm the important role of wild birds in *Campylobacter* epidemiology. Additional studies are required to investigate the potential role of wild birds in human campylobacteriosis.

Comparative Genomics of selected oral and intestinal *Campylobacter concisus* strains

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Campylobacter concisus is a fastidious bacterium of the human oral cavity, and is an emerging pathogen of the gastrointestinal tract. It is a heterogeneous species of phenotypically indistinguishable strains belonging to least two distinct genomospecies. In this study, comparative genomic analysis (CGA) was performed on four in-house sequences of oral and intestinal strains, assigned to genomospecies A (according to 23S rDNA typing), along with the genomes of *C. concisus* 13826 and ATCC 33237^T. Pairwise comparisons of the sequenced genomes with *C. concisus* 13826 showed a very high level of gene shuffling, while a high level of similarity and contiguity was observed with ATCC 33237^T. Two plasmids, 22 kb and 3.3 kb, were detected in only one of the sequenced genomes. The 3.3kb plasmid was a unique, high copy number plasmid with no similarity to other *C. concisus* genomes in the database. The pan and core genomes of the four sequenced strains along with *C. concisus* 13826 and ATCC 33237^T consisted of 2,790 and 1,463 protein coding genes, respectively. The clusters of orthologous groups (COG) of protein distribution patterns showed the involvement of more core genes in amino acid metabolism, transport; energy production, translation; ribosomal structure and biogenesis. Pan genes appeared to be involved in replication, recombination and repair, in addition to cell wall/membrane/envelope biogenesis related functions. This was also supported by the Blast2GO enrichment analysis, where the enrichment of RNA processes, and metabolic and biosynthetic processes were observed within the core genome and the pan genome enrichment was in defence responses and DNA-related processes. The genomic analysis of the sequenced genomes of oral and intestinal *C. concisus* strains indicated that there is a significant difference in gene content in these strains which could be related to the site of isolation rather than the genomospecies.

Epigenome-based adaptive evolution:
evidence from genomes, methylomes, transcriptomes
and phenotype in *H. pylori*

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The currently dominating model for adaptive evolution assumes selection from diverse genome sequences. However, diverse epigenomes may instead be regarded as the units of evolution as in cell differentiation in multi-cellular organisms. To examine this epigenome-based adaptive evolution model, we analyzed unicellular bacteria in which a somatic cell can be equated with a germ-line cell. In *Helicobacter pylori*, many of tens of DNA methyltransferases frequently change their sequence specificity by replacement of their Target Recognition Domains (TRDs).
We knocked out each of their specificity-determinant genes to analyze methylome, transcriptome and phenotype. Each knockout showed a unique effect on expression of specific genes for functional categories and on specific phenotype sets. Some methyltransferases affect expression of other methyltransferases and transcription factors forming a large network of gene expression. They control adaptive phenotypes such as motility, oxidative stress tolerance and DNA damage tolerance in a positive or a negative way.
The emerging picture of a dynamic network involving multiple ever-changing epigenetic systems and driving adaptation will provide a new paradigm in the study of adaptive evolution, we anticipate.

Pan-genomic analyses identify key *Helicobacter pylori* pathogenic loci modified by carcinogenic host microenvironments

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Helicobacter pylori is the strongest risk factor for gastric cancer; however, the majority of infected individuals do not develop disease. Pathologic outcomes are mediated by complex interactions among bacterial, host, and environmental constituents. Two dietary factors linked with gastric cancer risk are iron deficiency and high salt. We previously demonstrated increased gastric cancer rates among *H. pylori*-infected gerbils maintained on high salt or low iron diets. In the present study, we hypothesized that prolonged adaptation of *H. pylori* to *in vivo* carcinogenic microenvironments would result in genetic modifications important for disease. To test this hypothesis, whole genome sequencing of genetically related *H. pylori* strains that differ in virulence: B128, 7.13, and *in vivo* iron-adapted 7.13 output strains was performed. In addition, targeted *H. pylori* sequencing following prolonged exposure of bacteria to *in vitro* carcinogenic conditions was performed. A total of 180 unique SNPs were identified among the collective genomes when compared to a reference *H. pylori* genome. Importantly, common SNPs were identified in isolates harvested from iron-depleted and high salt carcinogenic microenvironments, including a SNP within *fur* (FurR88H). To investigate the direct role of low iron and/or high salt, *H. pylori* was continuously cultured *in vitro* under low iron or high salt conditions to assess *fur* genetic variation. Exposure to low iron or high salt selected for the FurR88H variant after only five days. To extend these results, *fur* was sequenced in a cohort of clinical *H. pylori* strains. Among the isolates examined, 18% (36/205) of strains from patients with premalignant and malignant lesions harbored the FurR88H variant, compared to 6% (8/124) of strains from patients with non-premalignant lesions ($P<0.005$). These results indicate that specific genetic variation arises within *H. pylori* strains during *in vivo* adaptation to conditions conducive for gastric carcinogenesis.

A large-scale comparative genomic survey of the *Campylobacter jejuni* population structure: towards the development of a nomenclature for global surveillance

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High-Throughput Sequencing (HTS) has the potential to revolutionize studies on the epidemiology of campylobacteriosis through the application of Whole-Genome Sequencing (WGS) in outbreak detection and molecular surveillance. Critically, this will require approaches for WGS-based subtyping and nomenclature for efficient tracking of subtypes of interest. We have performed a large-scale survey of the *C. jejuni* population using a prototype Core Genome MLST (cgMLST) scheme to examine optimal approaches for integrating population structure into a nomenclature for effective WGS-based surveillance.

Raw reads for 5,693 *C. jejuni* genomes from the SRA were assembled using the *INNUca* pipeline, annotated using *Prokka*, and analyzed using *Roary* to define a pangenome. Core genes with at least 99.9% carriage (n=697) were selected for a cgMLST scheme. Globally optimal eBURST (goeBURST) clusters were calculated from allele calls. Cluster membership was examined at a range of similarity thresholds and the Adjusted Wallace Coefficient (AWC) was used to assess cluster stability. The average distance between cluster members (i.e. intracluster distance) and distance to the nearest clusters (i.e. intercluster distance) was also examined.

Cluster analysis shows that similarity thresholds above 93.5% (i.e. 652/697 loci) yield clusters that are highly unstable and may be thus be unsuitable for long-term surveillance. Conversely, this threshold represents the first of several plateaux with significant cluster stability, in which clusters are characterized by short intracluster distances, large intercluster distances, and a unique complement of accessory genes and cgMLST alleles.

Core genome MLST will facilitate efficient analysis of WGS data given the exponential increase in WGS data from surveillance projects. Moreover, when coupled to a nomenclature based on clusters representing stable lineages in the population it will enable the tracking and monitoring of high-resolution subtypes of interest and the effective communication of WGS results to the public health, food safety, and research communities.

Integrated whole genome MLST and SNP analyses applied to publicly available Campylobacter jejuni/coli isolates

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Introduction: In laboratory-confirmed cases of infection, *Campylobacter* is considered to be one of the most significant bacterial causes of human gastroenteritis worldwide. Outbreaks have been traced to different food sources, although poultry is the largest source of outbreaks worldwide.
Presently, rapid and cost-effective generation and processing of whole-genome sequence (WGS) data offers a considerable advantage over traditional typing technologies and can considerably speed up detection and investigation of an outbreak.

Methods: Whole genome multilocus sequence typing (wgMLST) was applied to WGS data from about 1 000 publically available strains from clinical or poultry sources to detect clusters of highly related strains. Two independent allele calling approaches using BioNumerics, were applied, assembly-free and BLAST-based, to detect allelic variants in a quality-controlled manner.
One cluster defined by wgMLST was further characterized by whole-genome single-nucleotide polymorphism (wgSNP) analysis, tuned to reduce false positives while maximizing resolution by mapping the WGS reads to a high quality draft assembly chosen from within the cluster.

Results: wgMLST is suitable for the rapid analysis of large datasets, making it a useful technique for outbreak surveillance, while wgSNP can provide additional resolution increasing the confidence in detected clusters. In this case, it allowed us to quickly identify clinical cases related to poultry that might have previously gone unnoticed. Next to an integrated wgMLST and wgSNP analysis, BioNumerics also enables the validation of WGS analysis results against traditional data, rapidly providing a robust, portable and high resolution picture of molecular typing data.

Conclusion: The combination of two complementary approaches, wgMLST and wgSNP, on a virtually unlimited number of samples, managed by a single software platform opens many perspectives for food safety and public health monitoring programs. The BioNumerics solution combines the power of a cluster or cloud implementation with the ease of use of a local database and management software.

A stable and highly diverse genetic structure in clinical *Campylobacter jejuni* isolates in 2009 and 2015

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As the causal agent of the main foodborne disease in Europe, the development of *Campylobacter* genotyping methods raised in the last years improving the tracking of the pathogen. The standard method to characterize *Campylobacter* populations is Multilocus Sequence Typing (MLST). A new method adapted to routine analyses with a short execution time at a low cost, the Comparative Genomic Fingerprinting 40 (CGF40) has been developed by Taboada *et al*. (2012) and is based on the analysis of the accessory genome of *C. jejuni*.

Two collections of 143 and 371 clinical *Campylobacter jejuni* isolates from 2009 and 2015 were characterized using the CGF40. All the isolates were from human campylobacteriosis occurred in the 10 most populated departments in France. Isolates were categorized into types based on >90% CGF40 fingerprint similarity (CGF-90%). The obtained results were then compared to describe the evolution of the genetic structure of clinical isolates populations in the same locations at two different periods of time.

A high genetic diversity was observed within clinical isolates in 2009 and 2015 with Simpson's Diversity Indexes of 0.984 and 0.993, respectively. Six main CGF40-clusters were observed within the population from 2009 accounting for 70% of the isolates and four of them were also predominant in 2015 (32.9% of the population). However, one CGF40-cluster predominant in the population from 2015 was not observed in 2009 and included 4.6% of isolates. In addition, seven of the unique CGF40-clusters shown in 2009 were also shown in 2015, bringing the number of unique clusters to 52 representing approximately 10% of the global population.

Despite a high genetic diversity within both clinical populations of *C. jejuni* isolates, a stability of the genetic structure was observed with the similar predominant clusters in both populations, consistent with previously published work and the presence in 2015 of some unique clusters from 2009.

Campylobacter adaptation during acute and persistent human infections

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Zoonotic *Campylobacter jejuni* readily adapts to new host species for commensal colonization in birds and livestock or to cause disease in humans. Understanding adaptation during human pathogenesis can reveal how *C. jejuni* causes disease and can guide treatment design. Unfortunately, it is currently unclear how *C. jejuni* responds specifically to the human host. Beyond acute infections, the bacterial adaptations that contribute to recrudescent, persistent *C. jejuni* infections in humans are also not understood. Here we used a controlled clinical trial of human infection to characterize *C. jejuni* transcriptional and genetic adaptations *in vivo* during pathogenesis, along with a symptomatic non-human primate infection model to validate our approach. Variation in 11 *C. jejuni* genes is associated with either acute or recrudescent, persistent human infections which include gene products involved in host cell invasion, bile sensing, and flagella modification. *C. jejuni* from recrudescent, persistent infections exhibited significantly altered genomes that affect important transcriptional regulators and surface modifications. These data suggest therapy design should consider the intrinsic differences between acute and persistently infecting bacteria. Additionally, RNA-sequencing identified the *C. jejuni* transcriptome in human infection samples. Characterization of this adaption revealed a conserved transcriptional response during natural host commensalism and human pathogenesis. These advances inform therapy design, highlight pathogen adaptability across host species, and demonstrate the utility of multidisciplinary collaborations in future clinical trials to study pathogens *in vivo.*

Impact of immunisation and bottlenecks on phase-variable gene expression patterns during colonisation of chickens by *Campylobacter jejuni* strain

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Multiple genes of *Campylobacter jejuni* are subject to phase-variable ON-OFF switches in gene expression due to mutations in hypermutable repetitive sequences. The phase-variable genes encode outer membrane proteins and enzymes involved in addition of glycans and other moieties to the capsule, flagella and lipooligosaccharide. Switches in expression of these surface proteins and epitopes are hypothesised to mediate escape of host antibody responses and could contribute to poor outcomes in vaccine studies. We have previously reported on the switches in gene expression due to changes in the polyG/polyC tract lengths of 28 phase-variable loci as *C. jejuni* strain NCTC11168 colonises naïve chickens. In order to determine whether pre-existing immune responses influence the patterns of phase-variable gene expression, chickens were immunised with a whole cell lysate (WCL) or purified LOS of the homologous strain prior to challenge. Swabs samples were collected at 1, 14 and 28 days post-challenge and caecal samples at 28 days post-challenge. Detectable colonisation was delayed by immunisation with WCL at 14 days post-challenge but attained similar levels to non-immunised birds by 28 days post-challenge. As observed previously, temporal changes in PV expression states were observed between 14 and 28 days of colonisation in the control birds. At 28 days, only one gene (*cj0031*) exhibited major changes in the average expression levels between WCL-immunised and naïve birds while two other genes showed differences in median expression levels. Frequent bird-to-bird variation was observed in phase-variable gene expression states suggesting that heterogeneity due to bottlenecks may be the major driver of genetic variability rather than immune escape.

Could FlhF be a key element that controls *Campylobacter jejuni* flagella biosynthesis in the initial assembly stage?

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The disordered arrangement of flagella biosynthetic genes, combined with a simplified regulatory mechanism, has made elucidating the process of *Campylobacter jejuni* flagellation difficult. FlhF is a recently identified element that controls the assembly of the flagella, although how it functions is not clear at present. In this study, we found that inactivation of FlhF caused complete loss of flagella and associated motility in *C. jejuni*, and the transcription of most flagella genes was down-regulated. The importance of FlhF was systematically evaluated by analyzing changes in the transcription profiles between wild-type and flhF mutant starins, which showed that FlhF affects flagella biosynthesis from the initial assembly stage. FlhF is constitutively expressed during *C. jejuni* growth, demonstrating that it is a class I flagella element that participates in early flagella assembly. In addition, the early flagella component FlhB was not localized to the cell ploe in the *flhF* mutant. Thus, flagella assembly was impeded at the initial stage. We propose a model in which FlhF helps target the early flagella components to the cell pole, functioning prior to the formation of the flagella export apparatus, and thus places FlhF at the top of the flagella regulatory cascade hierarchy. Inactivation of FlhF impeded flagella assembly at the initial stage and decreased transcription of flagella genes through a feed-back control mechanism, leading to FlhF having a significant influence on the expression of late flagella components and resulting in the aflagellate *C. jejuni* phenotype. Our present study has uncovered how FlhF influences *C. jejuni* flagella biosynthesis, which will be helpful in understanding the *C. jejuni* flagella biosynthetic pathway and bacterial flagellation in general.

Genomic analysis of the recently emerged antimicrobial resistant *Campylobacter jejuni* ST-6964 in New Zealand

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Ciprofloxacin and tetracycline resistant *C. jejuni* ST-6964 spread rapidly and widely between poultry companies and humans in New Zealand following its first isolation in 2014. We studied the whole-genome sequences of 230 *C. jejuni* ST-6964 isolated from poultry and humans between 2014 and 2016, using both Illumina and PacBio sequencing. The genomic features of these isolates were compared at the nucleotide level, and also by considering the large-scale genomic influx of mobile elements, including genomic islands, phage insertions and plasmids. Phylogenetic analysis revealed signs of segregation into distinct clades strongly associated with the major NZ poultry suppliers. Human isolates admixed with poultry isolates in all clades, indicating human infections were linked to all major poultry suppliers. Comparative genomic studies provided evidence that, following the introduction of this sequence type into New Zealand, it has acquired and lost genes through multiple mechanisms including point mutation, conjugation, deletion, natural transformation, transduction, and simple sequence repeat variation. We also found evidence of multiple phage insertions mediated by a *tetO*-carrying plasmid. Some of the differences at the nucleotide level and large-scale genome influx seem random, whereas others correlate with host and poultry supplier. For example the *glcD* genes found in the human isolates associated with one poultry supplier were intact, whereas many of these genes in the poultry isolates in the same clade were truncated, suggesting that *glcD* gene truncation has happened multiple times in the clade associated with this poultry supplier, possibly as a result of selection. We hypothesize that the relatively low number of human cases associated with this poultry supplier may be linked to the observed *glcD* gene truncations. Our study highlights the high genomic plasticity and diversity of *C. jejuni* ST-6964 resulting in rapid, poultry company-associated evolution over a relatively short timescale.

Genome-wide association of disease severity traits in *Campylobacter* from malnourished children in the Peruvian Amazon

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Introduction: *Campylobacter* infection in developed countries peaks during infancy and again during early adulthood, with most infections being the result of consumption or handling of contaminated poultry products. In developing countries, the epidemiology of disease is quite different and *Campylobacter* infections are endemic among young children, especially those below 2 years old. Evidence from antibody analysis of people who have not reported campylobacteriosis symptoms, also hints at differences in disease presentation compared to developed countries, with asymptomatic infection common in adults and children, where infection can be associated with deficits in early childhood development.

Materials and methods: We quantitatively recorded campylobacteriosis symptoms including diarrhoea, fever and vomiting along with linked faecal samples collected as part of a screening program of 442 Peruvian children aged 0–72 months. 101 *Campylobacter* isolates were sampled and correlated with detailed disease severity records. Genome-wide association studies were used to identify genetic elements associated with asymptomatic carriage.

Results: Genetic elements were identified in 83 genes associated with severe disease. Functional filtering of SNPs, by correlation with clinical symptoms, implicated specific alleles in glycosylation, invasion and chemotaxis genes with haematochezia, the onset of fever and patient vomiting. Using a representative dataset of UK clinical isolates, we tested whether these alleles can be used to predict disease severity from European *Campylobacter* isolate genomes.

Conclusions: Characterisation of the genetic traits associated with disease severity and asymptomatic carriage of *Campylobacter* will inform intervention strategies against campylobacteriosis and the development of vaccines.

Homologous recombination between genetically divergent reptile-associated *Campylobacter fetus* lineages

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Campylobacter fetus shows a distinct intraspecific host dichotomy: *C. fetus* subspecies *fetus* (*Cff*) and *venerealis* (*Cfv*) are associated with mammals, whereas *C. fetus* subsp. *testudinum* (*Cft*) is primarily associated with reptiles. Recombination between these genetically divergent *C. fetus* lineages is extremely rare. It remained to be shown whether this apparent barrier to recombination was determined by the differential host preferences or by the genetic divergence between both lineages. These factors were studied in *C. fetus* ST69, a distinct strain which was obtained from a chelonian, yet closely related to mammal-associated *C. fetus*. Two isolates of *C. fetus* ST69 were obtained from the same individual chelonian. The genomes were sequenced using Illumina MiSeq and compared to the genomes of mammal- and reptile-associated *C. fetus* strains. Phylogenetic and recombination analysis was performed using Gubbins. With an average nucleotide identity (ANI) of 99.96%, the genomes of both isolates were highly similar. The ANIs between *C. fetus* ST69 and *Cff*, *Cft*, and *C. iguaniorum* were 98%, 92%, and 76%, respectively. The generally well-conserved *sapCDEF* genes, essential in formation of the *C. fetus* S-layer, were lacking in one of the isolates. In total, 5.1-5.5% of the core genome of both isolates showed signs of recombination. Of the 56 predicted recombination regions 80.4% were most closely related to *Cft*, 14.3% to *Cff*, and 5.6% (3/56) to *C. iguaniorum*. Two recombination regions were unique for one isolate, of which one was 100% homologous to *C. iguaniorum*. Recombination from ST69 to *Cft* was also detected, but to a lesser extent and only in chelonian-associated *Cft* strains. In conclusion, this study shows that the genetic divergence between *C. fetus* ST69 and *Cft* is no barrier to recombination when occurring in the same host type, and provides valuable insights in the dynamics of recombination and speciation in *C. fetus*.

Base-excising restriction enzymes ---
towards epigenetic immune systems

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All the restriction enzymes examined so far introduce DNA strand breaks by catalyzing hydrolysis of phosphodiester bonds linking the monomer nucleotide units. Here we report that one family of restriction enzymes represents not such phosphodiesterases but DNA N-glycosylases that excise a base from its recognition sequence.

Based on the behavior restriction-modification systems as mobile elements [Furuta, 2013], our genome comparison led to identification of a superfamily of restriction enzymes with a novel fold (Half Pipe) [Ishikawa 2005; Miyazono 2007]. Structural and biochemical analyses demonstrated that one of them excises the base to be methylated from its recognition sequence [Miyazono 2014]. The base excision is inhibited by methylation of the target adenine base.

At the resulting apurinic/apyrimidinic (AP) (abasic) site, its AP lyase activity generates atypical strand breaks [Fukuyo 2015]. The base excision is not coupled with the strand breakage and yet causes restriction as the restriction enzyme action can impair transformation ability of unmethylated DNA even in the absence of strand breaks *in vitro*. Bacterial AP endonucleases can introduce strand breakage at the AP site to promote such restriction [Fukuyo 2015] [Zhang 2016].

Its members are present in Campylobacter and Helicobacter. Distribution of this (PabI) family in global *Helicobcter pylori* strains reflects human migration: ancient movement of East Asian people to the Americas and recent movement of East Asian people and Indian people to Malaysia [Kojima 2015].

This surprising finding led us to generalize the concept of restriction-modification systems. Combination of any process of epigenetic DNA modification and any process of DNA damaging might form an epigenetic immune system. DNA uracil N-glycosylases may be regarded as one of them.

Poster session
« Pathogenicity and virulence factors »

Further investigation of the roles of fibronectin-binding proteins CadF and FlpA during *Campylobacter jejuni* interactions with intestinal epithelial cells

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The two highly conserved fibronectin-binding proteins CadF and FlpA play a role in *Campylobacter jejuni* adhesion to intestinal epithelial cells (IECs). Mutation of *cadF* or *flpA* in the two widely-studied *C. jejuni* strains 11168H and 81-176 reduced binding to fibronectin *in vitro* and also bacterial interactions with and invasion of Caco-2 and T84 IECs, however intracellular bacterial numbers increased over time between 3 and 24 hours. Mutation of *cadF* reduced the cytotoxicity of *C. jejuni* in the *Galleria mellonella* larvae model of infection to a greater extent than mutation of *flpA*. Both CadF and FlpA are associated with outer membrane vesicles (OMVs). OMVs isolated from *cadF* or *flpA* mutants are less immunogenic and cytotoxic than OMVs isolated from the wild-type strain. Using a 11168H strain expressing GFP at high levels, *C. jejuni* was shown to invade IECs, either residing within the *Campylobacter* containing vacuole (CCV), free within the cytoplasm and also in close proximity to the nucleus. Staining with a LAMP1 antibody revealed co-localisation with late endosomal compartments in parts of the trans-golgi network. *C. jejuni* infection of IECs leads to actin cytoskeleton rearrangements as seen by the formation of filopodia and lamellipodia, inducing membrane ruffling and formation of F-actin stress fibres after 24 hours infection. This is the result of activation of the small GTPase Rac1 following multiple signalling events after binding of CadF or FlpA to fibronectin on the surface of IECs. Mutation of *flpA* reduces Rac1 activation more than mutation of *cadF*. Pre-treatment with cytochalasin D (actin polymerisation inhibitor) or methyl-beta-cyclodextrin (caveolae-mediated endocytosis inhibitor) increases or reduces intracellular wild-type bacterial numbers respectively. The effect of different inhibitors on the ability of *cadF* and *flpA* mutants to invade IECs will be reported.

The *Campylobacter jejuni* protein Cj0588 as a model system for investigating the role of TlyA methyltransferases in bacterial cell

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Gene *cj0588* in the virulent *Campylobacter jejuni* strain 81176 encodes an ortholog of the protein TlyA that is also found in numerous bacterial pathogens. We show here that the protein Cj0588 is a TlyA-type I ($TlyA^{I}$) homolog that 2´-*O*-methylates 23S rRNA nucleotide C1920 in *C. jejuni.* Previous study indicated that the TlyA protein plays role in the colonization abilities of *C. jejuni, Helicobacter pylori* and *Brachyspira hyodysenteriae*. *C. jejuni* is also shown to form microcolonies and biofilms on human intestinal tissue and abiotic surfaces. In our studies we used the human pathogen *C. jejuni* 81176 strain with Cj0588 as an experimental model system for elucidating the role of TlyA in bacterial cell. We have engineered mutant *C. jejuni* 81176 strain in *tlyA* gene and strains with compelmetation in *tlyA* expressed TlyA protein from *C. jejuni* 81176 with and without RNA methylation abilities. Using those strains, we showed that changes in motility and biofilm formation are related to TlyA. We demonstrate decreased ability of biofilm formation and motility of strain with *tlyA* gene mutation which could have an influence on colonization ability and lead to reduction of virulence of *C. jejuni*.

Interactions between Dsb (disulfide bond) proteins of *Campylobacter jejuni* 81116

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Introduction: The bacterial proteins of the Dsb family catalyze the formation of disulfide bridges. The Dsb oxidative pathway of *Campylobacter jejuni* (CjDsb) differs from the well-characterized oxidative pathway of *E. coli.* The *E. coli* (EcDsb) oxidative pathway includes only two proteins, EcDsbA and EcDsbB. In C. *jejuni* 81116 the Dsb oxidative pathway consists of four extracytoplasmic proteins. Two are soluble periplasmic proteins (CjDsbA1 and CjDsbA2). The other two (CjDsbB and CjDsbI) are anchored in the inner membrane. CjDsbA1 and CjDsbA2 share a high degree of sequence identity.

Objectives: The goal of the presented work was to understand the *C. jejuni* Dsb network functioning.

Methods: To assess relations between Dsb proteins in *C. jejuni* cells we created double mutants in *C. jejuni* 81116 *dsb* genes by allelic exchange strategy. Autoagglutination and motility assays were performed on defined F12 medium, which does not contain cystine, to prevent non-specific oxidation of CjDsbA1.

Results: Previously conducted experiments showed that lack of CjDsbA1 results in unmotile and unable to autoagglutinate strain. The lack of CjDsbB and CjDsbA2 did not influence neither motility nor autoagglutination. Phenotypic analysis of the double mutated strains revealed unexpected data, two of them (*dsbA1dsbA2*, *dsbA1dsbB)* appeared to be motile and capable of autoagglutination in contrast to *dsbA1dsbI*.

Conclusions: The Dsb oxidative pathway of *Campylobacter* is more complex than that of *E. coli* and still needs deep analysis and more investigations to be fully understood. Our working hypothesis proposes that lack of both CjDsbAs results in changing cell's physiological conditions or activation the other protein which takes over DsbAs function. To verify this assumption phenotypic characterization of double mutated strains will be extended by performing PhoX (target of CjDsbA1) and Asst (target of CjDsbA2) activity tests .

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Helicobacter pylori Secreted Protein HP1286 Triggers Apoptosis in Macrophages via TNF-Independent and ERK MAPK-Dependent Pathways

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Introduction: Macrophages constitute a powerful line of defence against *H*. *pylori* infection. The final disease outcome is highly dependent on the bacterial ability to modulate the effector functions of activated macrophages. HP1286 is a secreted protein of *H. pylori*, suggested to play a role in bacterial colonization and persistence in the stomach as it was demonstrated to be overexpressed under acidic conditions.

Objective: Investigate the role of *H. pylori* secreted protein HP1286 in the regulation of macrophage responses.

Methods: HP1286 level of expression and release was investigated in eight different *H. pylori* strains. Primary human monocyte-derived macrophages (MDM) and macrophage cell lines (RAW 264.7, THP-1) were used for the cell assays. Binding assays and measurement of apoptosis were determine using LSR Fortessa flow cytometer. Caspase 3 activation was measured using the Caspase 3/7 activity assay. Activation of TNF and ERK MAPK signalling pathways were verified by immunoblotting.

Results: Exposure to rHP1286 induced apoptosis in macrophages in a time-and dose-dependent manner. Although interaction of rHP1286 was observed for several other cell types including human monocytes, differentiated neutrophil-like HL-60 and the T-lymphocyte Jurkat cell line, rHP1286 failed to induce apoptosis under similar conditions, indicating a macrophage-specific effect of the protein. A mutant strain of H. pylori lacking HP1286 protein expression was significantly impaired in its ability to induce apoptosis in macrophages. rHP1286 induced activation of caspase 3 and ERK MAPK signaling pathways. Additionally, nuclear translocation of ERK and phosphorylation of c-Fos was detected in macrophages treated with rH1286.

Conclusion: These results provide functional insight into the potential role of HP1286 during *H. pylori* infection. Considering the ability of HP1286 to induce macrophage apoptosis, the protein could possibly help in the bacterial escape from activated macrophages and persistence in the stomach.

The bile salt sodium taurocholate induces *Campylobacter jejuni* outer membrane vesicle production and increases OMV-associated proteolytic activity

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Campylobacter jejuni, the leading cause of bacterial acute gastroenteritis worldwide, secretes an arsenal of virulence-associated proteins within outer membrane vesicles (OMVs). *C. jejuni* OMVs contain three serine proteases (HtrA, Cj0511 and Cj1365c) which cleave the intestinal epithelial cell (IEC) tight and adherens junction proteins occludin and E-cadherin, promoting enhanced *C. jejuni* adhesion to and invasion of IECs. *C. jejuni* OMVs also induce IECs innate immune and antimicrobial defence responses. The bile salt sodium taurocholate (ST) is sensed as a host signal to co-ordinate the activation of virulence-associated genes in the enteric pathogen *Vibrio cholerae.* In this study, the effect of ST on *C. jejuni* OMVs was investigated. Physiological concentrations of ST do not have an inhibitory effect on *C. jejuni* growth until the early stationary phase. Co-culture of *C. jejuni* with 0.1% or 0.2% (w/v) ST stimulates OMVs production, increasing both lipid and protein concentrations. *C. jejuni* ST-OMVs possess increased proteolytic activity and exhibit a different protein profile compared to OMVs isolated in the absence of ST. ST-OMVs exhibit enhanced cytotoxicity and immunogenicity to T84 IECs and enhanced killing of *Galleria mellonella* larvae. Co-culture with ST significantly enhances the OMV-induced cleavage of E-cadherin and occludin. *C. jejuni* OMVs also cleave the major endoplasmic reticulum (ER) chaperone protein BiP/GRP78, mediated only by the protease Cj1365c. This data suggests that *C. jejuni* responds to the presence of the bile salt ST by increasing OMVs production and changing the protein content of OMVs to enhance pathogenesis.

Assembly of the Cytochrome *c* Oxidase in *Campylobacter jejuni*

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Campylobacter jejuni, uses complex cytochrome-rich respiratory chains, for growth and host colonisation, which includes a pathway to a cbb_3-type cytochrome-*c*-oxidase (CcoNOQP) with a very high oxygen affinity. This oxidase has been shown to be crucial in host colonisation and represents a potential anti-microbial target. The oxidase contains two c-type cytochrome subunits and a bi-nuclear haem-copper active site consisting of Cu(B) and b/b_3-type haems. Insertion of Cu(B) requires an assembly system of copper transporters and chaperones. In *C. jejuni* genes *cj0908 -cj0911* may encode a copper chaperone system. Proteins encoded by *ccoGHIS* play an important role in biogenesis of the cbb_3-type cytochrome-c-oxidase in other bacteria. In *C. jejuni*, we identified Cj1154 as CcoI-homologue, Cj1155 as CcoS-homologue and Cj0369 as potential CcoG-homologue. *Cj1485c and cj1486c* are directly downstream to CcoNOQP, with an unknown function. *cj1483c* may encode a CcoH homologue. We will present data on the roles of these genes in cbb_3-oxidase assembly. Oxidase activity is reduced in most of the mutants and no oxidase activity is measureable in *Δcj1155c* and *Δcj1486c* mutants but activity is restored in *Δcj1155c* on adding excess copper. Cu sensitivity assays showed that the growth defect of the *Δcj1155* mutant is reversed by increasing Cu concentrations. Our data suggest a model for the role of these proteins in the assembly of the oxidase, which will be useful in devising strategies for the inhibition of the oxidase itself or its assembly pathway.

Identification of *Helicobacter pylori* HP0231 (DsbK) targets by site-directed mutagenesis

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Introduction: The mechanism of disulfide bond formation in microorganisms is extremely diverse. Our recent work led to the characterization of the first dimeric oxidoreductase DsbK (HP0231)**.** It is intriguing that HP0231 acts as periplasmic oxidase, as EcDsbA, despite its structural resemblance to EcDsbG.

Objectives: To fully understand functioning of the Dsb machinery of *H. pylori* we decided to identify HP0231 substrates using point mutated forms of HP0231 and "reverse purification" substrates trapping strategy.

Methods: All genetic manipulations were performed using standard molecular biology procedures. Mutated forms of HP0231 were generated by site-directed mutagenesis and overexpressed in *E. coli* Rosetta strain. Substrate-HP0231 complexes were purified using Medium-Pressure Chromatography: mutated proteins with His-tag were immobilized on Ni-NTA column, then WT *H. pylori* 26695 lysate was loaded on column. At the end complexes were eluted with rising concentrations of imidazole.

Results: Two kinds of mutants were generated (P258T and C162S) by site directed mutagenesis. The P258T mutant slows the second step of oxidative folding, what should result in accumulation of its complexes with substrates. To trap the redox partner/s of HP0231 the C-terminal cysteine of CXXC catalytic site was replaced by serine. Lack the second cysteine of the catalytic motif disables resolving the disulfide complex. The mutated HP0231s with His-tag were immobilized on the column and reacted with *H. pylori* 26695 lysate. The eluted complexes were analyzed by SDS-PAGE, with or without reducing agent. Several additional bands appeared after reducing agent treatment, as compared to the non-reducing conditions. The proteins released from complexes will be analyzed by mass spectrometry.

Conclusions: Mutations in active CXXC site or cis-proline loop lead to create stable complexes of HP0231 with its substrates or redox partners and allow to identify proteins being HP0231 targets.

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C8J1298 – *Campylobacter jejuni* dimeric oxidoreductase – oxidase or isomerase?

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Introduction: Bacterial Dsb enzymes are involved in formation of disulfide bonds between cysteine residues. The Dsb protein network has been well characterized in cells of the model microorganism *Escherichia coli*, but little is known about its functioning in other bacteria species. *Campylobacter jejuni* Dsb oxidative pathway is composed of four proteins - two periplasmic DsbA's and two inner membrane proteins DsbB and DsbI. Recently C8J1298 has been identified as potential component of *C. jejuni* Dsb system by bioinformatic analyzes.

Objectives: The aim of presented work was to characterize C8J1298 protein by *in vitro* experiments.

Methods: All genetic manipulations were performed using standard molecular biology methods. Correctness of the obtained constructs were confirmed by sequencing. C8J1298 protein was overexpressed in *E. coli* Roseta strain and purified using NGC™ Medium-Pressure Liquid Chromatography System. Purified protein was used to conduct biochemical assays: insulin reduction assay, izomerization/oxidation RNase activity test and analysis by gel filtration chromatography.

Results: *In silico* analysis indicated that C8J1298, apart from catalytic domain with thioredoxin fold contains also dimerization domain. So, it was classified as a member of Dsb oxidoreductases displaying isomerizing activity. Determined biochemical features of C8J1298 confirmed previous predictions. Insulin reduction assay demonstrated that C8J1298 displays high reductase activity of similar level as EcDsbC. Also its both, oxidizing and isomerizing activities were comparable to those of EcDsbC. Gel filtration chromatography showed that C8J1298 exists as a homodimer. To get a complete biochemical characterization of C8J1298 we intend to check its oligomeric state by glutaraldehyde crosslinking and solve its structure by crystallography.

Conclusions: Presented results showed that C8J1298 belongs to the family of Dsb proteins and probably acts as isomerase.

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Impact of the selected amino acids, N- and C-terminal parts of HP0377 on its functioning as CcmG (cytochrome c maturation) protein

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Introduction: *Helicobacter pylori* HP0377 protein is a Dsb thiol oxidoreductase which acts as CcmG (cytochrome c maturation) protein. HP0377 resolved structure is similar to that of other CcmG proteins, and HP0377 reduces the oxidized form of apocytochrome c *in vitro*. In contrast to many CcmGs HP0377 exists as a mixture of monomeric and homodimeric forms, and is essential for cell viability.

Objectives: The biochemical properties of Dsb oxidoreductases are conditioned by the presence of highly conserved motifs: the CXXC active site within the TRX fold and the *cis*-proline loop. The aim of this study was to establish the link between the amino acid residues of active motifs, N- and C-terminal parts of HP0377 and its ability to reduce apocytochrome c and its oligomeric state.

Methods: Site-directed mutagenesis of *hp0377* were carried out using a QuickChange mutagenesis kit. Deletions within *hp0377* coding sequence were generated by standard strategy and confirmed by sequencing. DNA fragments were cloned into *E. coli* expression vector. Subsequently, wild type HP0377 and its variants were purified by affinity chromatography and used for *in vitro* assays.

Results: We found that HP0377 variants with cysteine of the CXXC motif changed to alanine do not reveal ability to reduce apocytochrome. HP0377 variant in which threonine in *cis*-proline loop was replaced with valine, acts as a wild type version of protein. All HP0377 point mutated versions similarly to wt HP0377 are able to generate homodimeric forms. The two HP0377 truncated variants lacking C- or N- fragments are able to form homodimeric forms and to reduce apocytochrome c.

Conclusions Ability to reduce apocytochrome c is determined by active site CSYC but not by *cis*-proline loop. The mechanism of HP0377 oligomerization still remains inexplicable.
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Investigation of the role of *Campylobacter jejuni* Type VI secretion system in secretion of effectors and interactions with host cells

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The Type VI Secretion System (T6SS) is a contact-dependent secretion machinery and contraction of the TssB and TssC contractile sheath translocates the TssD inner tube out of the cell and across the target membrane, delivering effectors that interact with both bacterial and host cells. The role of *C. jejuni* T6SS is not yet well elucidated. Recent studies suggest that T6SS may be more prevalent in both human and chicken isolates than previously indicated. Previous studies indicate that *C. jejuni* T6SS has a role in adherence and invasion of human epithelial and murine macrophage cells, also in haemolysis of red blood cells. Most of the widely studied *C. jejuni* wild-type strains do not contain a T6SS. The *C. jejuni* 488 wild-type strain is a recent human isolate. Genome sequencing revealed all 13 genes associated with T6SS are present and TssD expression in the 488 strain was confirmed using a TssD antibody. Investigation of the role of the *C. jejuni* T6SS in secretion of effectors and interactions with host cells has been initiated by mutagenesis of *tssB*, *tssC* and *tssD* in the 488 strain, as well as another human isolate 43431. Preliminary comparison of the phenotypes of these mutants to the wild-type strain revealed that the 488 *tssC* mutant exhibited decreased haemolysis of red blood cells. Analysis of whether T6SS function is abolished in *C. jejuni* when either *tssB* or *tssC* are mutated is being investigated by detecting the presence of secreted TssD. The effects of host factors such as bile salts and mucin on the expression of T6SS genes will be reported as well as the abilities of the different *tss* mutants to adhere to and invade human intestinal epithelial cells. In addition, the interactions between T6SS-positive and T6SS-negative *C. jejuni* strains will also be reported.

Campylobacter jejuni infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses

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Introduction: *Campylobacter jejuni* is among the leading bacterial agents causing enterocolitis worldwide. Despite the high prevalence of *C. jejuni* infections and its significant medical and economical consequences, intestinal pathogenesis is poorly understood. This is mainly due to the lack of appropriate animal models. In the age of 3 months, adult mice display strong colonization resistance (CR) against *C. jejuni*. Previous studies underlined the substantial role of the murine intestinal microbiota in maintaining CR.

Methodology/Results: Due to the fact that the host-specific gut microbiota establishes after weaning, we investigated CR against *C. jejuni* in 3-week-old mice and studied intestinal and extra-intestinal immunopathogenesis as well as age dependent differences of the murine colon microbiota. In infant animals infected orally immediately after weaning *C. jejuni* strain B2 could stably colonize the gastrointestinal tract for more than 100 days. Within six days following infection, infant mice developed acute enterocolitis as indicated by bloody diarrhea, colonic shortening, and increased apoptotic cell numbers in the colon mucosa. Similar to human campylobacteriosis clinical disease manifestations were self-limited and disappeared within two weeks. Interestingly, long-term *C. jejuni* infection was accompanied by distinct intestinal immune and inflammatory responses as indicated by increased numbers of T- and B-lymphocytes, regulatory T-cells, neutrophils as well as apoptotic cells in the colon mucosa. Strikingly, *C. jejuni* infection also induced a pronounced influx of immune cells into extra-intestinal sites such as liver, lung, and kidney. Furthermore, *C. jejuni* susceptible weaned mice harbored a different microbiota as compared to resistant adult animals.

Conclusion: These results support the essential role of the microbiota composition in CR against *C. jejuni* and demonstrate that infant mouse models resemble *C. jejuni* mediated immunopathogenesis including the characteristic self-limited enterocolitis in human campylobacteriosis. Furthermore, potential clinical and immunological sequelae of chronic *C. jejuni* carriers in humans can be further elucidated by investigation of long-term infected infant mice. The observed extraintestinal disease manifestations might help to unravel the mechanisms causing complications such as reactive arthritis or Guillain-Barré syndrome.

Blood adaptation mechanisms of *Campylobacter jejuni* associated with systemic infection

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A highly pathogenic *Campylobacter jejuni* clone, named SA (for sheep abortion), has recently emerged as the major cause of Campylobacter-associated sheep abortion in the U.S. It is also transmitted to humans via raw milk and other unknown routes, causing food borne gastroenteritis. The virulent hallmark of clone SA is its ability to induce bacteremia and systemic infection, and eventually abortion in pregnant animals. Although we have made progresses in understanding the pathogenic mechanisms of clone SA, how *C. jejuni* adapt in blood remains poorly understood. We performed a time-course transcriptome analysis of *C. jejuni* clone SA survival using an ex vivo model of sheep whole blood infection. We observed that *C. jejuni* clone SA altered the expression of ~36% (600/1666) of its ORFs. The major dynamic changes in expression were associated with genes involved in transcription and translation, amino acid transport and metabolism, inorganic ion transport and metabolism, chaperones, cell wall/membrane biogenesis, energy production and conversion, signal transduction mechanisms, and cell motility. Through mutagenesis studies of a subset of up-regulated genes, five novel virulence factors were identified to be important for survival in sheep blood. One of them, cjsa_0039, which encodes a GTP-binding protein (typA), was demonstrated to play an important role in abortion induction in the guinea pig model. These results identified new virulence factors involved in pathogenesis and abortion induction, and provide new insights into how *C. jejuni* adapts in blood during systemic infection.

C. coli clade 3 strains induce cell death in human HT-29 colon cells

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We previously characterized *C. coli* strains of clades 2 and 3 that were originally isolated from surface water treatment plants in Sweden, using comparative genomics and phenotypical analyses. Here we studied the ability of these environmental isolates to infect human cells using an *in vitro* infection model. All clade 3 water strains and the clade 3 clinical strain 76339 had a cytotoxic effect on the cells, which showed clear signs of necrosis with membrane disruption, swelling and finally cell ruption, already two hours after infection. Clade 3 strains adhered better to human HT-29 colon cancer cells shortly after inoculation, while adhesion of the clade 2 water strains was higher later on during infection. There was no difference in the induction of IL-8 by these strains. Phylogenetic analyses of known virulence genes showed that clade 2 strains were more closely related to clade 1, than clade 3 strains. Moreover, CiaB, CadF and IamA proteins showed a high degree of clade 3-specific amino acid sequence variations. Analyses of expression levels by quantitative RT-PCR revealed that the clade 3 isolates had lower expression levels of the *cadF*, *iamA* and *ceuE* genes than the clade 2 isolates, while there was no difference in the expression levels of *cdt*, *ciaB* and *pldA* between the clades. Together, these results support our previous speculations that *C. coli* clade 2 strains would be more adapted to mammalian hosts and the efficient progression of infection and expression of virulence genes, shown in the present study, would ensure spread of colonization. In contrast, one would expect clade 3 strains to be less successful in causing infection due to their rapid killing of cells. However, it remains to be studied how the clinical clade 3 strain 76339 has overcome the toxic effect to cause the verified human infection.

Campylobacter virulence in pathophysiology of post infection Irritable Bowel Syndrome

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Background: Campylobacter enteritis is associated with post-infection irritable bowel syndrome (PI-IBS). Our aims were to determine: 1) the point prevalence of PI-IBS; 2) clinical risk-factors for PI-IBS development; and 3) virulence patterns of PI-IBS causing and control (PI no-IBS) strains.

Methods: 1737 Campylobacter cases were surveyed 6-9 months following notification for current and pre-infection IBS symptoms. Univariate analysis was done to compare distribution of variables and logistic regression modelling to evaluate risk factors for PI-IBS development. We studied C. jejuni adhesion and invasion using the gentamicin protection assay. IL8 induction was measured in vitro. A $p<0.05$ was considered statistically significant.

Results: Of the 500 patients (excluding pre-infection IBS), 121 (24%) met the IBS criteria; mostly IBS-mixed and diarrhea (109/121). PI-IBS subjects were younger and more likely to be female. Compared to those without subsequent PI-IBS, more PI-IBS patients reported vomiting, diarrhea ≥7 days, and hospitalization, and fewer reported fever during enteritis. Antibiotic use was similar between the two groups. Multivariate analysis showed increased risk with: female gender (odds ratio [OR], 95% CI: 2.0, 1.3-3.3, $p<0.001$), hospitalization (3.0, 1.6-5.7, $p<0.001$), duration of diarrhea (1.5, 1.2-1.9, $p<0.001$), and vomiting (1.7, 1.1-2.9, $p=0.029$). Older age (0.97, 0.95-0.98, $p<0.001$) and fever (0.5, 0.3-0.8, $p=0.007$) were inversely associated. Mean (SEM) bacterial adhesion {2.2 x 10-3 (3.3 x 10-4) vs 1.7 x 10-3 (4.9 x 10-4), $p=0.03$}, invasion {2.5 x 10-4 (4.2 x 10-5) vs 1.5 x 10-4 (4.3 x 10-5), $p=0.003$} and IL8 induction {61.2 (5.8) vs. 38.6 (3.8), $p<0.005$} was greater with PI-IBS than control strains (n=29/group).

Conclusion: Fever during enteritis was inversely associated; however, younger age, female gender, vomiting, hospitalization and diarrhea lasting ≥ 7 days were positively associated with Campylobacter PI-IBS. PI-IBS causing *C. jejuni* strains have greater *in vitro* virulence. Unique host-pathogen interactions likely play a role in pathophysiology of PI-IBS.

Phase-variable epigenetic gene regulation modulates *Helicobacter pylori* motility and virulence factor expression

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Helicobacter pylori is one of the most genetically diverse bacterial pathogens in existence due to high mutation and recombination rates. *H. pylori* strains also have an extraordinarily large number of DNA methyltransferases compared to other bacteria; the *H. pylori* genome is consequently highly methylated in a strain-specific manner, thus resulting in extensive epigenetic interstrain diversity. The *H. pylori* phase variable gene *modH,* typified by gene HP1522 in strain 26695, encodes a N^6-adenosine type III DNA methyltransferase. We previously identified multiple strain-specific *modH* variants (*modH1 – modH17*) and showed that phase variation of *modH5* in *H. pylori* P12 influenced expression of motility-associated genes and outer membrane protein gene *hopG*. However, the ModH5 DNA recognition motif and the mechanism by which ModH5 controls gene expression were unknown. We used comparative single molecule, real-time sequencing to identify the DNA site methylated by ModH5. We found that this motif is vastly underrepresented in *H. pylori* genomes, but overrepresented in a number of virulence and outer membrane protein genes. In line with our previous observation that key motility genes were transcriptionally regulated by *modH5* ON/OFF status, we observed that motility of *H. pylori* P12 wild-type (*modH5* ON) was significantly higher than that of isogenic *modH5* OFF or Δ*modH5* mutants, indicating that phase-variable switching of *modH5* expression plays a role in regulating *H. pylori* motility. Using the *flaA* gene, which encodes flagellin A, as a model, we determined that ModH5 modulates *flaA* promoter activity in a GACC methylation-dependent manner. These findings not only confirm the role of ModH5 in gene regulation but also provide a novel insight into how ModH5 mediates epigenetic regulation of *H. pylori* motility.

cj0371: a novel virulence-associated gene of *Campylobacter jejuni*

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Campylobacter jejuni is the major cause of human bacterial diarrhea worldwide. Its pathogenic mechanism remains poorly understood. cj0371 is a novel gene that was uncovered using immunoscreening. There have been no previous reports regarding its function. In this study, we constructed an insertion mutant and complement of this gene in *C. jejuni* and examined changes in virulence. We observed that the cj0371 mutant showed significantly increased invasion and colonization ability. We also investigated the role of cj0371 in motility, chemotaxis and growth kinetics to further study its function. We found that the cj0371 mutant displays hypermotility, enhanced chemotaxis and enhanced growth kinetics. In addition, we localized the Cj0371 protein at the poles of *C. jejuni* by fluorescence microscopy. We present data that, collectively, significantly proves our hypothesis that cj0371 is a new virulence-associated gene and through the influence of chemotaxis plays a negative role in *C. jejuni* pathogenicity.

Helicobacter suis induces changes in gastric inflammation and acid secretion markers in pigs of different ages

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Helicobacter suis is a zoonotic agent that is highly prevalent in the stomach of pigs. The mechanisms involved in its persistence in the stomach and in its effect on gastric acid secretion were studied, by measuring the impact of a *H. suis* infection in 2-3 months old, 6-8 months old and adult pigs on mRNA expression of markers for gastric acid secretion (H+/K+ ATPase, KCNQ1, gastrin, CCK-B receptor, somatostatin, Sonic Hedgehog, H2 receptor, M3 receptor) and inflammation (IL-4, IL-8, IL-10, IL-17A, IL-1β, IFN-γ, CXCL-13) in gastric tissue. A possible association with ulceration in the non-glandular part of the porcine stomach was determined.

An increased prevalence of *H. suis* and a shift of colonization towards the fundic gland zone in adult sows was shown, while the number of *H. suis* bacteria per mg tissue decreased with the pigs' age. During the more acute phase of the infection, an innate immune response was detected. A Treg response in combination with decreased expressions of IL-8, IL-17A and IFN-γ was indicated to be present in the *H. suis*-infected 6-8 months old pigs, which may have contributed to persistence of *H. suis*. In *H. suis*-infected adult pigs, a Treg response accompanied by a Th17 response was indicated, which may have played a role in the decreased number of *H. suis* bacteria in the stomach of this age group. While no clear alterations in the markers for gastric acid secretion were detected in 2-3 months old pigs, a decrease and increase were found in 6-8 months old pigs and adult sows, respectively. Presence of severe hyperkeratosis and erosions in the non-glandular part of the stomach was only seen in the *H. suis*-positive groups.

These results show that *H. suis* infection affects the expression of markers for gastric acid secretion and inflammation and indicate that these effects differ, depending on the infection phase.

Poster session
« Immunology and Host response »

The intracellular proteins of *Helicobacter pylori* trigger immunomodulatory response in gastric epithelial AGS cells

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The inflammatory response is critical process in acute and chronic *Helicobacter pylori* infection leading to peptic ulcer, gastric adenocarcinoma, gastric mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer. *H. pylori* colonization in the human gastric epithelial cells induces the secretion of interleukin-8 (IL-8), which is a potent PMN-activating chemotactic cytokine. The aim of this study was to elucidate the mechanism of intracellular proteins of *H. pylori* in activating interleukin-8 (IL-8) and neutrophil migration. The soluble protein of *H. pylori* was prepared in PBS by sonication, passed through a 0.20-mm filter and determined protein concentration. The human gastric adenocarcinoma cells (AGS) were cocultured with the soluble protein extract at various concentrations. The level of IL-8 secretion was examined after induction by intracellular proteins of *H. pylori* compared with alive organism at various time intervals using ELISA. The boyden chamber assay was performed to investigate the leukocyte migration. *H. pylori* presented the IL-8 activation as same as its intracellular proteins. The level of IL-8 secretion was increased in time dependent manner at 6, 12, 24 and 48 h. The soluble protein extracted from *H. pylori* induced the neutrophil and monocyte migration. This study provides evidence that proteins localized within *H. pylori* play a major role in immunomodulatory of both CC and CXC chemokines activation. To characterize those intracellular proteins affecting the immune response might be a new target for developing therapeutic approach for *H. pylori* infection.

Investigation of the Influence of Phase Variation on the Biological Phenotypes, Immunity to and Structure of the Flagella in *Campylobacter jejuni*

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Campylobacter jejuni is a commensal of chickens that is responsible for many cases of food borne gastroenteritis in humans. *C. jejuni* produces bipolar flagella comprised of two structural proteins FlaA and FlaB, which are modified with as many as 19 *O*-linked glycans. The flagellum appears essential for colonization of the chicken gastrointestinal tract and is also a major antigen that elicits protective immunity. Some of the *O*-linked glycans are synthesised by biosynthetic enzymes that undergo phase variation through slipped-strand mispairing in G/C-tracts. Currently, the function of protein glycosylation in *C. jejuni* is unknown but it may contribute to evasion of the host immune response. Therefore, flagellar filament protein (FlaA) would be an excellent target for further investigation. We generated a non-glycosylated FlaA protein by cloning the flaA gene into an expression vector (pLEICS-1) followed by expression in a bacterial expression system and purification of recombinant FlaA protein by His tag affinity chromatography. Recombinant FlaA was compared with modified FlaA protein, in whole cell lysates of *C. jejuni*, for reactivity with sera from chickens challenged with *C. jejuni* by Western blotting. Reactivity to FlaA proteins increased with time of colonisation. Higher reactivity with recombinant FlaA than with glycosylated FlaA was also noted. No differences were observed in reactivity to the glycosylated form of the protein between two *C. jejuni* strain NCTC11168 variants, a chicken-adapted variant (Ca11168) and hypermotile variant (H11168). We hypothesise that these antibody screening results provide a rationale for the modification of filament flagella with sugar moieties influencing immune recognition by the host. Future experiments will focus on testing whole cell lysates and FlaA proteins derived from mutant strains of selected phase variable genes involved in the modification of FlaA protein to understand the contributions to immune reactivity of specific glycan structures.

Vaccination with a capsule conjugate or infection with *Campylobacter jejuni* induces serum bactericidal antibodies measured using a flow cytometric-based assay

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Campylobacter jejuni is among the most common causes of diarrheal disease worldwide and efforts to develop protective measures against the pathogen are ongoing. One of the few defined virulence factors targeted for vaccine development is the capsule polysaccharide (CPS). We have developed a prototype capsule conjugate vaccine against a HS23/36 CPS (81-176) that is immunogenic in multiple pre-clinical animal studies, previously shown to be protective in a non-human primate challenge model (Monteiro *et al*. Infect Immun. 2009. Mar;77(3):1128-36) and moderately immunogenic in a Phase 1 clinical study. Because other licensed capsule conjugate vaccines against encapsulated gram-negative organisms induce bactericidal antibody responses, which are used as a correlate of protection, we developed a flow cytometry-based assay to measure serum bactericidal activity (SBA) against *C. jejuni*. We demonstrated that SBA responses are generated in mice, rabbits, and non-human primates immunized with the CPS conjugate vaccine and more recently, we have developed this assay to evaluate functional antibodies in sera from humans immunized with a CPS conjugate. Some volunteers that generated a serum anti-CPS IgG response also generated functional SBA antibody responses indicating that anti-CPS antibodies may play a protective role against *C. jejuni*. In addition to vaccine-mediated responses, we were also able to measure SBA activity in volunteers that were challenged with *C. jejuni* strain CG8421. Due to the difficult microbiological conditions and costs required for *C. jejuni* culture-based SBA assays, this flow cytometry-based SBA assay is more straight-forward, can be adapted to high-throughput, cost-effective analysis of clinical specimens and strains and should facilitate analyses of functional antibodies against multivalent *C. jejuni* capsule conjugate vaccines.

Kaempferia parviflora inhibits inflammatory response mediated by _Helicobacter pylori_ in human gastric cells

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Background: *Helicobacter pylori* is known as a carcinogenic to human gastric cell. Other than that, host immune response triggered by *H. pylori* is another factor of the cancer development. Inflammation plays a vital role in the early stage of *H. pylori* pathogenesis which many *H. pylori* virulent factors can massively activate the immune responsiveness in host gastric cells. Neutrophil is the first inflammatory cell responder. They are migrating to infection site by Interleukin 8 attraction which released from *H. pylori* infected gastric cell. In this study, we tested *Kaemperia parviflora* extracts activity to inhibit inflammation process.

Method: Human gastric cells (AGS cell) were infected with *H. pylori* ATCC 43504 and co-culture with *K. parviflora* extracts at 8 and 16 µg/ml. IL-8 levels in the co-culture supernatants at 6, 12, 24 and 48 hours were examined by using ELISA. HL-60 cell was differentiated to neutrophil by 1.25% DMSO for determined anti-neutrophil chemotaxis effect, both of neutrophil and KP extract were applied in the upper chamber of 5 µm membrane transwell in which IL-8 was used for inducing neutrophil migration toward lower chamber of transwell.

Results: 16 µg/ml of *K. parviflora* reduced gastric IL-8 secretion effectively at 6 and 12 hours. However, both of *K. parviflora* extracts at 8 and 16 µg/ml significantly inhibited neutrophil chemotaxis mediated IL-8 attraction. DMSO which used to dissolve the extract was tested as a vehicle control and interference was not found in both experiments.

Conclusion: *K. parviflora* is prominent for the alternative prevention and therapy of *H. pylori* infection through inhibit IL-8 secretion and neutrophil chemotaxis which are the major activities in inflammatory process.

Genomic origin for the variability of host-immune response triggered by *Helicobacter pylori*

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Introduction: *Helicobacter pylori* is present in about 50% of the global population. Numerous studies have shown a link between *H.pylori* and gastritis, gastric ulcers, and gastric cancer which is the 5th most common cancer. Nevertheless, large numbers of people remain asymptomatic despite *H. pylori* colonisation. Investigation of strains where clear clinical diagnoses have been made is now critical to understand *H. pylori* infection biology. *H. pylori* from clearly defined clinical groups will be compared for their genomic variability and effects on host cells.

Methods: We performed a co-culture experiment of 15 clinical strains of *H. pylori* isolated from patients suffering from gastric cancer or mild gastritis, on two types of cells associated with *H. pylori* pathogenesis. AGS cells were used to study the effect of *H. pylori* infection on epithelial cells, and differentiated THP-1 cells were used as a model for macrophages. Supernatants were harvested for cytokine analysis by ELISA.

Results: Assay of mediators confirmed the role of CagPAI for IL-8 production in AGS cells, and identified further *H. pylori* genes potentially linked to smaller but significant variations in cytokine production.

Conclusion: While the host's immune response to every pathogen is different, we have identified unique responses within a clinically defined group of *H.pylori*. Defining the different immune responses to commensal and pathogenic *H. pylori* and the genes responsible for these differences will help clinicians to develop precision medicine for infectious disease.

Evidence for extra-intestinal spread of *Campylobacter* spp.; analysis of bird matched samples

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Campylobacter spp. has a long association with poultry and for many years it was considered to be a gut commensal. However, with the detection of *Campylobacter* in other organs such as the liver, the bacterium can no longer be considered a true commensal of poultry. The aim of this study was to ascertain if there were differences in *Campylobacter* strains colonising the gut or liver of commercially reared poultry.

Methods: The liver, ileum and caecum were removed from 30 birds at the time of slaughter, from the same commercial flock reared in the UK. Liver samples were enriched in modified Exeter broth and gut samples were serially diluted and plated onto mCCDA. Colonies were purified onto COLBA and DNA extracted using a DNA blood and Tissue Kit. Samples were WGS sequenced at the Swansea Genome Centre.

Results: *Campylobacter* was isolated from 100% of the liver samples (using enrichment culture), 73% of the caecal samples and 83% of the ileal samples (using direct plating). MLST and WGS indicated that the liver isolates were more related to ileum isolates rather than those from caecum. However, both *C. jejuni* and *C. coli* were isolated from all 3 sample locations. Strikingly, genes that were present in the liver and ileal isolates were absent in those from the caecum.

Conclusion: This study shows that there are differences in the carriage of *Campylobacter* in the liver and gut within the same chicken. The close relationship between liver and ileum isolates, compared to the caecum, suggest that extra-intestinal spread occurs from the ileum. The results obtained implicate important 'invasion' elements shared at the *genera* level as *C. jejuni* and *C. coli* were present in the flock.

Diverse Responses of Human and Chicken Intestinal Epithelial Cells to a bacterial population within the species *Campylobacter jejuni*

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Background: *Campylobacter* remains the major cause of gastroenteritis-associated food poisoning in the Western World causing a significant burden to health services. *Campylobacter* are particularly diverse, with a broad host range and significant genetic recombination within and between species. The response of chickens and humans to *Campylobacter* is different with the former able to tolerate particularly large loads while the latter remains particularly sensitive.

Aim: To investigate the diversity in *Campylobacter* strains from different sources and sequence types. *C. jejuni* species, were used to investigate in inflammatory, cytotoxicity, invasion and signalling responses in avian and human intestinal cell lines.

Method: *Campylobacter* isolates (130) from multi-locus sequence typed collections were cultured on blood free selective medium and incubated at 42^{o}C in microaerophilic conditions. Standardised aliquots of *C. jejuni* strains were used to infect intestinal human HT-29 cells and Avian 8E11 cells prior to determination of inflammatory (qPCR), cytotoxicity (alamar blue), adhesion and invasion (gentamicin protection) responses.

Results: *C. jejuni* induced IL-8 and CXCLi1 (and CXCLi2) in human and avian epithelial cells respectively in a MAP kinase dependent manner. In contrast, IL-10 responses in both cell types were PI-3-kinase / akt dependent. *C. jejuni* induced diverse invasion responses in human and avian epithelial cells. High invasion was dependent on MAP kinase signalling in both cell lines. *C. jejuni* induced diverse cytotoxic responses in both human and avian epithelial cells with *cdt* positive isolates showing significantly higher toxicity.

Conclusions: These results clearly demonstrate that each *Campylobacter* strain has its own unique infection biology in both humans and avian epithelial cells. Such phenotypic diversity should be considered important when designing strategies to negate the deleterious effects of *C.jejuni* on the food industry.

Campylobacter colonisation in chickens: extra-intestinal spread linked to stress?

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Campylobacter has a well-documented relationship with poultry. Increasing cases of human campylobacterosis are being attributed to the consumption of chicken livers. *Campylobacter jejuni* and *C. coli* are capable of leaving the chicken gut to colonise other organs, including the liver. Previous reports suggest that *Campylobacter* invasion *in vitro* is increased in the presence of noradrenaline which leads to the hypothesis that stress during the end of a chicken's life could lead to extra-intestinal spread of *Campylobacter* to other tissues.

Chickens were experimentally infected with *Campylobacter* isolates that were either "invasive" (isolated from chicken livers) or "non-invasive" (isolated from the gut). Following colonisation at 21 days old, chickens were reared under conditions to mimic commercial practices. At 37 days old the control group were culled with minimal stress, the harvest group underwent all practices that a bird would commercially (including feed withdrawal, catching, transport, lairage, shackling and stunning) and the final group were injected with 6-hydroxydopamine (experimental stress). Birds from all groups were culled at defined time-points, blood samples were taken along with the liver, caecum and ileum, where *Campylobacter* counts were estimated in each tissue. Sections of the ileum were stored in RNA later for histopathology.

There were differences in *Campylobacter* colonisation in the gut and liver between treatment groups despite high variation within each group. Bird serum cortisol (stress) was partly related to commercial practices and increased leading up to final stunning. Extra-intestinal spread did not appear to be associated with serum cortisol.

This commercially relevant study has identified important factors in the extra-intestinal spread of *Campylobacter* from the gut and include; the strain of *Campylobacter,* the importance of stress markers used, and changes in the local microbiota at the bird level.

Poster session
« Antibiotics and antimicrobial resistance »

What affects antimicrobial resistance in *Campylobacter* along the broiler chicken supply chain?

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Introduction: Human campylobacteriosis is a major enteric disease; resistant infections can be more severe and harder to treat thus increasing disease burden. Poultry is a major source of *Campylobacter* and use of antimicrobials in poultry production is scrutinized for its impact on resistance. This study aimed to describe resistance in *Campylobacter* isolates from broiler chicken in Canada and to test associations with select risk factors. A secondary objective was to use the results of this study to validate the sampling scheme and outputs of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS).

Methods: Samples were collected across Canada in 2013 using a stratified, weighted random sampling at 3 points along the supply chain: caeca samples at slaughter, carcasses at processing and raw chicken at retail. All *Campylobacter* isolates were tested for resistance to 9 antibiotics by broth microdilution. Logistic regression was used to explore associations between antimicrobial resistance (AMR) and five factors: point of origin, season, province, *Campylobacter* species, and data origin (CIPARS vs. current study).

Results: Among 1,460 isolates tested, 53% were resistant to at least one antimicrobial. The most common AMR profiles were tetracycline (39%), both quinolones and tetracycline (6.6%), and quinolones only (3.5%). Overall, *Campylobacter coli* showed more resistance than *C. jejuni* and significant differences were observed between provinces. Other variables were not associated with AMR and no marked difference in AMR was observed between the data from this study and CIPARS.

Conclusion: This study provides evidences that resistance in *Campylobacter* from raw chicken at retail originated further upstream in the supply chain, and highlights the importance of action at the farm level to reduce *Campylobacter* resistance and improve food safety and public health. The comparability of CIPARS with the current study validates the CIPARS sampling scheme and provides evidence for the validity of its outputs.

Antimicrobial resistance and characterization of *gyrA* mutation of *Campylobacter jejuni* and *Campylobacter coli* through commercial broiler production chains in Thailand

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Contaminated poultry meat is regarded as the main source of human campylobacteriosis which is a major cause of diarrhoeal diseases worldwide. The aims of this study were to determine antimicrobial resistance level and *gyrA* mutation in *C. jejuni* and *C. coli* isolated from breeders and broilers in Thailand. Thirty-six *C. jejuni* and 94 *C. coli* isolates collected through two broiler production chains during September 2014 and February 2015 were tested by two-fold agar dilution for their susceptibility to antimicrobial agents. Most *Campylobacter* isolates were multidrug resistant (MDR), defined as being resistant to three or more antimicrobial classes (*C. jejuni*: 100%; *C. coli*: 98.9%), and exhibited high resistance to enrofloxacin (*C. jejuni*: 100%; *C. coli*: 98.9%). The vast majority of *C. coli* were resistant to tetracycline (97.9%), trimethoprim-sulfamethoxazole (81.9%), and doxycycline (79.8%), but only 55.6%, 36.1%, and 50% of *C. jejuni* isolates revealed resistance to these antimicrobial agents, respectively. In contrast, all isolates were susceptible to both erythromycin and gentamicin. A selected subset of 24 *C. jejuni* and 24 *C. coli* were characterized for their mutations in the quinolone resistance determining region (QRDR) of the *gyrA* gene by nucleotide sequence analysis. The Thr-86-Ile substitution (ACA-ATA in *C. jejuni* or ACT-ATT in *C. coli*) was found in all isolates. Furthermore, Arg-6-Ser (AGC-AGG), Gln-7-Lys (AAA-CAA), Ser-22-Gly (AGT-GGT), Asn-203-Ser (AAT-AGT) and Ala-206-Val (GCA-GTA) mutations were all detected for the first time in enrofloxacin-resistant *C. jejuni*. In conclusion, the emergence of MDR and high resistance rates to several antimicrobials are major concerns identified in this study. The prudent use of these agents and active surveillance of resistance at the farm level are essential steps to reduce the public health risks identified in this work.

Comparative analysis of virulence and antimicrobial resistance of genetically different *Campylobacter* from poultry farms

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Introduction: Despite the importance of *Campylobacter* as a foodborne pathogen, the exact mechanisms by which *C. jejuni* or *C. coli* cause infection are unknown. Analysis of the virulence gene and antimicrobial resistance of different PFGE patterns was applied among strains isolated from poultry farms.

Material and Methods: A total of 21 PFGE patterns (19 corresponded to *C. jejuni* and 2 corresponded to *C. coli*) were analyzed to identify *cadF, flaA, cdtA, cdtB,* and *cdtC* virulence genes using conventional PCR. Likewise, six antimicrobials were tested: ciprofloxacin, erythromycin, azithromycin, gentamicin, tetracycline and nalidixic acid. European Committee on Antimicrobial Susceptibility Testing (EUCAST) cut off values were used for the interpretation of the MIC (Minimal Inhibitory Concentration). *C. jejuni* strain ATCC 33560 was used as a control.

Results: All the *C. jejuni* PFGE patterns presented *flaA*, *cadF*, *cdtA*, *cdtB* and *cdtC* gene*;* however, neither *cdtA* nor *cdtC* genes were detected in *C. coli* patterns. Likewise, all of *C. jejuni* patterns were resistant to ciprofloxacin, tetracycline and nalidixic acid, with the exception of pulsotype F that was sensitive to all antibiotics except to tetracycline. *C. coli* patterns (T and U) showed higher resistance than *C. jejuni* patterns. They were classified as multi-resistant because at least, they were resistant to four different classes of antibiotics.

Conclusion: The fact that *C. coli* patterns showed an absence in some subunit of the cytolethal distending toxin (*cdtA* and *cdtC*) could explain the high percentage of campylobacteriosis caused by *C. jejuni* than by *C. coli*. Moreover, higher antimicrobial resistances were observed in *C. coli* patterns. This study pointed out that efforts must be taken in the first steps of the poultry processing chain to avoid that virulence and high antimicrobial resistance *Campylobacter* spp. isolates caused infection in consumers.

Multilocus sequence typing of multiresistant *Campylobacter coli* strains isolated from turkey in Poland

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Campylobacter is currently a leading cause of human bacterial gastroenteritis in the Europen Union. A wide genetic diversity of *Campylobacter* population in poultry has been reported in different studies performed with a variety of genotyping methods. Most of the investigations focused on *C. jejuni* and the population structure of *C. coli* is not well known.
In the present study Multilocus Sequence Typing (MLST) was used to analyse the genetic relatedness of multiresistant *C. coli* isolated from turkey.
The study was performed on 28 *C. coli* selected out of 118 isolates from turkey ceca that were examined for antimicrobial resistance (AMR) profile using the microbroth dilution method with 6 antimicrobials: gentamicin (GEN), streptomycin (STR), erythromycin (ERY), nalidixic acid (NAL), ciprofloxacin (CIP), and tetracycline (TET). The epidemiological cut-off values according to EUCAST were applied. The isolates resistant to at least three different classes of antimicrobials were analysed with MLST. The primer sets for DNA amplification and sequencing of housekeeping genes (*aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt*, *uncA*) were from the *Campylobacter* MLST database. The obtained data were analysed for Sequence Types (STs) and clonal complexes.
The study revealed 17 different STs which five were novel (8628, 8629, 8630, 8631, 8632). Fifteen of STs belonged to clonal complex CC 828 and two to CC 1150. The most frequent ST was 1055 (6 isolates) followed by ST 3017 (4 isolates). Thirteen STs were represented by single isolates. Ten different STs were observed among 17 isolates with the most prevalent AMR profile CIP, NAL, TET, STR. *C. coli* isolates of four different STs were resistant to CIP, NAL, TET, ERY, whereas six strains with four STs displayed the CIP, NAL, TET, STR, GEN, ERY resistance profile.
The results of this study revealed a considerable genetic diversity among multiresistant *C. coli* of turkey origin in Poland.

Diversity of *tet*(O)-like determinants in *Campylobacter* spp isolated from Pigs in Scotland.

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Introduction: *Campylobacter* species are the major cause of foodborne bacterial gastroenteritis in humans. This study examined the role of the pig host reservoir in human campylobacteriosis in Grampian, Scotland from 2012 – 2015 and also characterised the abundance of tetracycline resistance determinants.

Materials and methods: A total of 219 *Campylobacter* isolates were cultured from pig faecal samples. The DNA extracted and whole genome sequencing was performed on an Illumina HiSeq 2000 sequencer with 100 base paired-end sequencing and the FASTQ reads were assembled using Velvet. The genomes were assessed for purity and submitted to Bacterial Isolate Genome Sequence Database (BIGSdb) where allele were tagged. All the isolates were typed by multi-locus sequence typing. For isolates other than *Campylobacter jejuni* /*coli*, *atpA* typing was used for speciation. Source attribution modelling was performed using a STRUCTURE with alleles Model and the Asymmetric Island Model. The CAMP1698 locus (tetracycline resistance determinant) was characterised in all isolates and a phylogenetic tree constructed using different tetracycline resistance alleles.

Results: *Campylobacter coli* [51% (n=112)] and *Campylobacter* spp. [49% (n=107): *C. hyointestinalis, C. ignotis, C. lanienae, C. lari*] were isolated from pig faecal samples. Source attribution modelling attributed a pig source to 0% to 8% of contemporaneous clinical *C. coli* isolates. 85% of *C. coli* isolates and 72% of *Campylobacter* spp. isolates had the tetracycline resistance determinant. The predominant CAMP1698 alleles in *Campylobacter* spp. were 34, 75 and 191 and in *C. coli* were 4 and 93 and these were all *tet*(O) variants, however chimeric *tet*(O/M/O) and *tet*(O/32/O) variants were found in some strains.

Conclusion: *Campylobacter* from pigs is not a major source of human infection in Scotland but it is a reservoir of tetracycline resistance determinants. The spread of tetracycline resistance determinants by horizontal gene transfer has likely shaped the evolution of these variants.

Campylobacter jejuni isolated from pigs and cattle in Poland: genetic diversity and antibiotic resistance profiles

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Pigs and cattle are underestimated source of *Campylobacter* infection in humans. Limited data is available on sequence types (STs) and antimicrobial resistance (AMR) of *C. jejuni* among pig and cattle isolates in Poland.

The objective of this study was to determine genotypes and AMR profiles of *C. jejuni* to better understand the diversity of Polish population of this pathogen.

A total of 44 *C. jejuni* strains were isolated from 529 bovine and 277 pig carcasses from 2009 to 2016. The isolates were typed by multilocus sequence typing (MLST) and their resistance to 6 antimicrobials was determined using the MIC method.

Amongst 22 isolates from cattle 18 STs were identified with ST22 represented by four strains. The predominant clonal complex (CC) CC22 was detected in seven *C. jejuni* from this source. Similar situation concerning the genetic diversity was observed among 22 isolates from pigs. Overall, 17 STs were identified, including ST50 and ST403 detected in two isolates of each STs. The most frequent CC403 was observed among five strains. Four novel STs (three from cattle and one from pig) were recognized among the isolates tested. The most common AMR profile observed among 10 strains from cattle and 10 from pigs was ciprofloxacin, nalidixic acid and tetracycline. Only one *C. jejuni* showed resistance to three classes of antimicrobials tested: quinolones, aminoglycosides and tetracyclines and it was of pig origin.

A high genetic diversity of the *C. jejuni* isolates was demonstrated in this investigation and the obtained typing data did not correlate with antimicrobial resistance phenotypes. The molecular analysis of the isolates showed that they were mainly of the same MLST types as those previously deposited in the MLST database.

Interconnection of antibiotic resistance, genetic proximity and the origin of *Campylobacter jejuni* isolates

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Campylobacteriosis is one of the main concerns of public health. It is the most common bacterial cause of gastroenteritis in the developed countries. Most of the cases are self-limiting and do not require a therapy intervention. However, hospitalization and intensive antibiotic treatment is often necessary when immunocompromised patients, newborns and elderly people are infected, as the disease can lead to severe dehydration. Increasing resistance of campylobacters to antibiotics leads to inefficient therapy resulting in further complications. The aim of this study was to investigate possible spread of antimicrobial resistance among Campylobacter jejuni isolates, with regard to their origin and genetic proximity. The susceptibility profiles of the isolates were determined by disk diffusion method and evaluated according to EUCAST guidelines. In total, 64 isolates originated from clinical samples and raw chicken meat were examined. Of these, 22 isolates were resistant to ciprofloxacin, 1 to erythromycin and 12 to tetracycline. In total, 9 isolates were multiresistant with one isolate resistant to all of the three examined antibiotics. The phylogenic relationship was resolved by multiplex PCR binary typing (mP-BIT).

Molecular characterisation of tetracycline and ciprofloxacin resistant *Campylobacter jejuni* strains isolated from humans, poultry and wild birds

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Background: *Campylobacter jejuni* is the leading cause of gastroenteritis worldwide challenged with increasing antibiotic resistance due to their misuse and overuse misuse in humans and animals.

Objectives: The aim of this study was to screen for presence of *tet(O)* gene and the point mutations in the quinolone resistance determining region (QRDR) of the *gyrA* gene of *C. jejuni* strains isolated from humans, poultry and wild birds.

Methods: In total 292 *C. jejuni* strains from infected children (n=100) broiler products (n=96) and wild birds (n=96) were tested for *tet(O)* gene and for point mutations in the QRDR of *gyrA* gene by sequencing. The DNA sequences were analysed with Bioinformatics software suite (BioNumerics 7).

Results: The *tetO* gene was found in 72% of tetracycline-resistant *C. jejuni* strains. The Thr86Ile (ACA→ATA) *gyrA* mutation was the single polymorphism detected in all strains from broiler products and in 98 out of 100 from children strains resistant to ciprofloxacin. Interestingly, eight *C. jejuni* strains had three novel mutations: Arg48, Glu136 and Ala at position 122. The sequence analysis of *gyrA* revealed that 13 of the 15 *C. jejuni* strains (86.6%) with MICs ≥128 μg/ml for ciprofloxacin had two missense mutations i.e. Thr86Ile (ACA→ATA) and Ser22Gly (AGT→GGT). The *gyrA* gene point mutations A64G, G118T and C257T were observed in four and two strains assigned to ST-257 (CC257), ST-51 (CC443) and ST-6413 (CC353) MSLT genotypes isolated from infected children and broiler products, respectively.

Conclusions: Resistance to tetracycline conferring *tet(O)* gene was widely present in *C. jejuni* strains despite the isolation origin. The majority of quinolones resistant *C. jejuni* strains (77%) carried the Thr-86-to-Ile mutation in the *gyrA* gene. In most cases two missense mutations Thr86Ile (ACA→ATA) and Ser22Gly (AGT→GGT) were associated with elevated resistance of *C. jejuni* strains to ciprofloxacin.

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The heterogeneous response of isogenic *C. jejuni* NCTC11168 to erythromycin

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Introduction: Heterogeneous responses of bacterial populations to antibiotic exposure have been extensively reported in scientific literature. Although the importance of the phenomenon is not fully understood, it may have impact on diagnostic-testing and medical treatments and contribute to resistance development.

Materials and methods: Population analysis profiling (PAP) was used to evaluate population heterogeneity in resistance to erythromycin. Decimal dilutions of exponentially growing *C. jejuni* NCTC11168 cells were spotted on agar plates containing different concentrations of erythromycin (0, 1, 2, 4, 8, 16 and 32µg/ml) and CFU/ml are determined, after 2-5 days of incubation (depending on the erythromycin concentration) under microaerobic conditions at 42°C. WGS was also employed.

Results: While the majority of the population was able to grow in the presence of 1µg/ml erythromycin, a sub-population able to grow at 4µg/ml occurred at a Frequency=$2.8 \times 10^{-4} \pm 2.3 \times 10^{-4}$. PAP analysis with single colony isolates (SCIs) from 4µg/ml plates showed that the whole populations retained the ability to grow at 4µg/ml erythromycin concentration following unselective cultivation. No single nucleotide polymorphisms, gene-amplifications or common phase-variable states could be detected for the SCIs from 4µg/ml compared to the SCIs from 0µg/ml erythromycin. Additionally, a small part of the population of the SCIs from 4µg/ml concentration was able to grow on 8µg/ml erythromycin containing plates (Frequency=10^{-5}-10^{-6}), a concentration under which no growing sub-population was observed for the parental strain. This indicates that the occurrence of sub-populations may lead to a gradual increase of resistance levels and may contribute to resistance development.

Conclusion: Sub-populations able to grow on plates containing 4µg/ml erythromycin occurred at high frequencies in *C. jejuni* populations and have a stable phenotype. Part of the population of SCIs from 4µg/ml erythromycin plates can grow under 8µg/ml erythromycin implying that the observed heterogeneity may contribute to resistance development.

A case of recurrent multidrug resistant *Campylobacter jejuni* bacteraemia in an immunocompromised host– clinical presentation and management

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Introduction: *Campylobacter* spp. can cause persistent infection in the immunocompromised host. We present a case of recurrent multidrug resistant *Campylobacter jejuni* bacteraemia and the challenges encountered in management.

Methods and Results: 40 year old Caucasian man with common variable immunodeficiency was empirically treated for diarrhoea in December 2015 with cefixime and azithromycin, later stool was found to be *Campylobacter* PCR positive. In September 2016 he developed worsening diarrhoea; *Campylobacter jejuni* was isolated from stool and blood. WGS confirmed the identification and data mining for common antimicrobial resistance determinants performed.
C. jejuni isolated from stool and blood had the following MICs (mg/L) : erythromycin > 256, ciprofloxacin >32, tetracycline 32, ertapenem 0.5, co-amoxiclav 16. Additionally meropenem MIC was 0.5, tigecycline 0.032, gentamicin 0.5 and chloramphenicol 2 mg/L on the blood isolate. He was treated with ertapenem 1g daily (for 14 days and then 16 days) and subsequently meropenem 1 g 8 hourly for 3 weeks with adjunctive oral chloramphenicol (500mg qds). However, he continued to fail treatment, 3 further blood cultures showed increase in MIC of meropenem to 16, while MICs of tigecycline, gentamicin and chloramphenicol remained unchanged. He finally attained clearance after treatment with a combination of tigecycline (8 weeks), chloramphenicol (7 weeks in total) and gentamicin (10 days).
WGS revealed presence of tet(O) and mutations in gyrA , 23S, L22 and L4 ribosomal genes that explained resistance to tetracycline, ciprofloxacin and erythromycin. One amino acid change (K45E) in the *cmeB* gene, substitution in the ribosome binding site of blaOXA-61 and alterations in PorA were identified and likely to be associated with resistance to carbapenems in later isolates.

Conclusion: Secondary resistance due to prolonged antibiotic use is a problem in immunosuppressed patients with *Campylobacter.* Absence of established breakpoints makes it difficult to select appropriate antibiotics for treating multidrug resistant infections.

Campylobacter jejuni and *Campylobacter coli* prevalence and antimicrobial susceptibility pattern in travelers' diarrhea in Thailand and Nepal

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Background: *Campylobacter jejuni* (CJ) and *C. coli* (CC) infections are associated with acute gastroenteritis in humans and a leading cause of travelers' diarrhea (TD). Antimicrobial resistance of *Campylobacter* to empirical diarrhea treatment has been increasingly reported worldwide. This study aims to describe prevalence of campylobacteriosis in travelers with diarrhea and non-diarrhea controls in Nepal and Thailand and to describe the antimicrobial susceptibility pattern.

Methods: Etiological studies of TD were conducted in Thailand and Nepal during 2012-2014. Stool cultured on blood agar with filtration under microaerophilic incubation was used for isolation and *Campylobacter* was identified by biochemical testing. The disk diffusion and E test techniques were applied for antimicrobial susceptibility testing.

Results: In Thailand, CJ and CC were detected significantly more in 43/171 (25%) and 9/171 (5%) of cases as compared to controls of 5/165 (3%) for CJ and 0% for CC. In Nepal, CJ was detected in 76/414 (18%) of cases and 10/209 (5%) in controls ($p<0.001$) and CC was detected in 3% of cases and 1% in controls. Antimicrobial susceptibility testing of CJ in Thailand (N=48) and Nepal (N=87) showed resistance to ciprofloxacin (92% and 97%), erythromycin (0% and 5%) and azithromycin (0% and 5%), respectively while CC in Thailand (N=9) and Nepal (N=17) showed resistance to ciprofloxacin (89% and 100%) and azithromycin (33% and 24%), respectively. Focusing on CC isolates from Thailand, none of the isolates were fully susceptible to nalidixic acid (100% resistance) or ciprofloxacin (11% with intermediate susceptibility and 89% resistance) and 33% were resistance to azithromycin.

Conclusions: CJ is an important cause of TD in both Thailand and Nepal. Despite lower prevalence as compared to CJ, the high level of resistance of CC to ciprofloxacin and azithromycin, the first and second line drug for treatment of campylobacteriosis, has made treatment options limited.

Typing and resistance characteristics of *Campylobacter jejuni* and *Campylobacter coli* isolates from patients in Germany

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Introduction: The number of *Campylobacter* infections reported in Germany is increasing since 2007 (https://survstat.rki.de). Between 2005 and 2016, the National Reference Centre (NRC) for *Salmonellae and Other Bacterial Enterics* at the Robert Koch Institute, Wernigerode Branch, tested 2,096 *Campylobacter jejuni* and 1041 *Campylobacter coli* isolates from stool specimens of diarrhoea patients obtained from primary labs for susceptibility to 11 antimicrobials. Since 2012 discrimination of major capsular types by PCR was introduced and 881 *Campylobacter jejuni* were analysed.

Material and Methods: The antibiogram (MIC determination by means of the broth microdilution test according to CLSI directions) was created as an epidemiological marker for pathogen isolates with epidemiological cut-off (ECOFF) values of EUCAST (http://mic.eucast.org/Eucast2/) as well as based on DIN values for *Enterobacteriaceae* and, for some antimicrobials, based on the preliminary MIC_{90} values for all *Campylobacter* spp. isolates tested so far at the NRC.
Multiplex PCR for determination of *cps*-Genes was performed according to Poly *et al*. (2011) J. Clin. Microbiol., 49(5):1750.

Results: Majority of tested *Campylobacter* strains were multidrug-resistant. 68% of the *C. jejuni* and 96% of the *C. coli* isolates tested were resistant to at least three antimicrobials. The prevalence of co-resistance to ciprofloxacin + erythromycin, to ciprofloxacin + gentamicin and to erythromycin + gentamicin was observed to increase.
Dominant *cps* types were HS 4 (14%), CG8486 (12.6%; including mixed type HS 4 / CG8486: 7.3%) and HS 2 (11.5%).
Two small outbreaks relevant to *cps* type HS 4 and one to HS 2 were observed.

Conclusion: Data of resistance situation and results for subtyping of major capsular types of *Campylobacter* reflect the situation in Germany. Regional differences, for example between rural regions with large-scale livestock farming and big cities, cannot be excluded. Continuous evaluation of data from a largely consistent population over this period allows an assessment of trends.

A pediatric case of enteritis associated with a *Campylobacter coli* strain resistant to gentamicin: Study of the resistance associated genes

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The genus *Campylobacter* is the leading cause of diarrhea worldwide. Gentamicin resistance in *Campylobacter* was first detected in USA from a single human isolate of *Campylobacter coli* in 2000 and since then, this resistance has increased rapidly representing 12.2% of human isolates in 2011. A recent study performed in USA has investigated the molecular epidemiology of gentamicin-resistant *Campylobacter* and the authors concluded that this resistance was acquired by horizontal gene transfer. Furthermore, they discovered several new genes associated with gentamicin resistance. The aim of the present study was to screen for the presence of several of these genes by PCR in a gentamicin resistant strain involved in a case of pediatric diarrhea.

The strain from diarrheic stool of a 1-year-old female child with acute enteritis was isolated in Campylosel agar (bioMérieux). Antibiotic resistance was evaluated by disk-diffusion with 10 µg gentamicin disks. No inhibition halo was obtained. Genetic identification was performed with the *rpoB* gene. The strain was screened by PCR for the presence of four resistance associated genes i.e. aacA4, aac(6')-Ie/aph(2'')-Ia (also named aacA/aphD, encoding a bifunctional enzyme), the aph(2'')-If and aph(2'')-Ig.

The strain was identified as *Campylobacter* sp. on the basis of the Gram stain and a positive hippurate test. Genetic identification confirmed the strains as *Campylobacter coli*. Of the 403 strains identified at the hospital as *Campylobacter* spp., between 2013-2016, 10 (2.4%) showed resistance to gentamicin, but this resistance was more common in 2016 (5.3%). Among the four genes studied only two were detected, the aph(2")-If and the bifunctional enzyme aac(6')-Ie/aph(2")-Ia.

Resistance to gentamicin in *Campylobacter* is a rare presentation found to be increasing in our hospital. Screening for this resistance and for the involved genes will help to determine its prevalence and importance.

Acquisition of second mutation in *gyrA* caused high resistance to sitafloxacin in *Helicobacter pylori* after unsuccessful sitafloxacin containing treatment

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Introduction: Sitafloxacin and amoxicillin triple therapy is promising rescue treatment for *H. pylori* eradication. However, a part of strains were not eradicated by sitafloxacin and amoxicillin triple therapy. Evaluation of resistance for sitafloxacin and amoxicillin in the survived strains is important for planning strategy to overcome multidrug-resistant strains after unsuccessful eradication.

Material and methods: Patients who failed sitafloxacin and amoxicillin triple treatment as a third-line therapy were enrolled at Keio University Hospital between December 2011 and March 2015. *H. pylori* isolates were obtained from gastric biopsy specimens before and after the treatment. The minimum inhibitory concentrations of sitafloxacin and amoxicillin against *H. pylori* isolates were determined with the agar dilution method. *GyrA* mutation status of *H. pylori* was also analyzed. Penicillin breakpoints of 0.06 to 0.5 mg/ml (intermediate category) and 1 mg/ml (resistant category) and sitafloxacin breakpoints of 0.12 to 1 mg/ml (intermediate category) and 2 mg/ml (resistant category) were employed.

Results: 17 patients were enrolled. Before the treatment, incidences of resistant, intermediate and susceptible strains to sitafloxacin were 5.9%, 88.2% and 5.9%, respectively. In all strains *gyrA* were mutated, 82.4% were N87-only mutants, and 17.6% with D91 only. There was no double mutation detected in *gyrA* prior to the treatment. Incidences of resistant, intermediate and susceptible strains to amoxicillin were 0%, 70.6% and 29.4%, respectively. 17.6% acquired second mutation in *gyrA* after the treatment. As a result, rate of resistance to sitafloxacin increased from 5.9% to 17.6%. Meanwhile, no strains obtained resistance to amoxicillin after the treatment.

Conclusion: Acquisition of second mutation in *gyrA* caused high resistance to sitafloxacin in *H. pylori* after unsuccessful eradication with sitafloxacin, amoxicillin and proton pump inhibitor triple regimens. On the other hand, no strains obtained resistance to amoxicillin.

Diversity of *Helicobacter pylori* clarithromycin resistance in gastric mucus samples

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Introduction: To detect the real status of *Helicobacter pylori (H. pylori*) resistance to clarithromycin in the stomach.

Material and Methods: Gastric endoscopic mucus samples collected by endoscopic brushes from 49 urease breath test positive patients in Henan Cancer Hospital were gradually diluted by brain heart infusion and cultured on Karmali agar plates at 37°C in micro-anaerobic conditions (5% O_2, 10% CO_2 and 85% N_2). 16 isolates, or all the isolates if total isolate number was below 16, were randomly selected from each sample. Clarithromycin E-test were performed on each isolate.

Results: 563 isolates were successfully collected in 39 out of 49 samples, among which 4 samples only grows 1 to 12 isolates. Totally, there were 22 and 6 samples are all sensitive(S) and all resistant(R) to clarithromycin. Notably, in other 11(28.9%) samples, 16 isolates disaccorded on clarithromycin susceptibility, ranged from 1/16 to 15/16 resistant. Additionally, MIC of each isolates varies considerably (e.g. from 0.19 to >256 ug/ml in one sample) were the widest fluctuation in the all sensitive, all resistant or R/S mixed samples.

Conclusion: Great diversities of *H. pylori* clarithromycin susceptibility in the stomach were showed, and brings out a great challenge to the traditional method of clarithromycin susceptibility examination. Novel approaches shall be introduced to cover the whole picture of *H. pylori* clarithromycin susceptibility in the stomach.

Mechanism of resistance to β-Lactams in *Helicobacter cinaedi*

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Helicobacter cinaedi is the most prevalent enterohepatic *Helicobacter* species in humans and can cause nosocomial infections. Several antimicrobial agents, including β-lactams, are used to treat *H. cinaedi* infections; however, a standard treatment regimen has not been established. Here, we isolated the MRY12-0051 strain from Japan, which exhibited high-level resistance to ceftriaxone. To elucidate the mechanism of antimicrobial resistance, the MRY12-0051 genome sequence was compared to the β-lactam susceptible strain MRY08-1234 with a shared sequence type (ST10). Specifically, 70 non-synonymous SNPs were found in 60 genes in MRY12-0051, including penicillin-binding protein genes (2 mutations in *pbpA*, 1 mutation in *pbp2*, and 2 mutations in *ftsI*). Transformation and penicillin binding assays revealed that mutations in *pbpA* and *ftsI* conferred a significant increase in ceftriaxone resistance. Additionally, the *H. cinaedi* genome encoded several efflux pumps, including RND-type efflux pumps. Quantitative RT-PCR for expression analysis of these pumps in MRY12-0051 indicated a 11 to 25-fold increase compared to that observed in the *H. cinaedi* strain CCUG18818, whereas MRY08-1234 showed a 4 to 18-fold increase. While attempts to knockout genes in MRY12-0051 failed repeatedly, knockout of *cmeB* and *cmeD* encoding RND-type efflux pump components in CCUG18818 resulted in respective decreases of 8- and 64-fold in ceftriaxone MIC. We previously reported MIC_{90} value of amoxicillin in *H. cinaedi* isolates from Japan as 8 μg/ml. On the other hand, amoxicillin MIC in most clinical *H. pylori* isolates from Japan is reported to be under 0.125 μg/ml. Hence, the difference in β-lactam susceptibilities of *H. cinaedi* and *H. pylori* would be associated with the activity of efflux pumps. Collectively, these data indicated that *pbpA* mutations are the primary mechanism responsible for ceftriaxone resistance in *H. cinaedi*, however significant increases in efflux pump expression in certain strains may also facilitate β-lactam resistance.

Poster session
« Emerging and Related Organisms species »

Rewriting the rulebook: minimal standards for describing new *Campylobacter*, *Arcobacter*, *Helicobacter* and *Wolinella* species

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The foundation for accurate identification of taxa is a sound classification. Ongoing changes in taxonomic methods, and in the rapid development of the taxonomic structure of species assigned to the Epsilonproteobacteria have lead the International Committee of Systematic Bacteriology Subcommittee on the Taxonomy of *Campylobacter* and Related Bacteria to discuss significant updates to previous minimal standards for describing new *Campylobacter, Arcobacter, Helicobacter,* and *Wolinella* species. The core underlying principle remains the use of appropriate phenotypic and genotypic methods to characterise strains sufficiently so as to effectively and unambiguously determine their taxonomic position in these families, and provide adequate means by which the new taxon can be distinguished from extant species and subspecies. This polyphasic taxonomic approach demands the use of appropriate reference data for comparison to ensure the novelty of proposed new taxa, and the recommended study of at least five strains to enable species diversity to be assessed. . Methodological approaches for phenotypic and genotypic (including whole-genome comparisons) characterisation are recommended. Metrics for whole-genome comparisons are presented. These standards aim to help the wider scientific community to characterise, describe and identify these important organisms.

The role of emerging Epsilonproteobacteria in childhood gastroenteritis

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Each year billions of children experience acute gastroenteritis globally, resulting in over a million deaths. Current laboratory methods fail to find an aetiological agent for up to 80% of faecal samples. The bacterial class Epsilonproteobacteria contains several species known to cause gastroenteritis but the role many more species play in this illness is unclear. To better understand this relationship, a Multiplex Ligation-dependent Probe Amplification (MLPA) assay for the detection of 28 taxa within the Epsilonproteobacteria was developed and applied to a collection of DNA extracts from a Belgian childhood gastroenteritis case control study. Using culture-based methods, Epsilonproteobacteria were isolated from 17.4% (32/184) of cases and 5.1% (9/175) of controls. *Campylobacter jejuni* was isolated from 26 cases and 3 control, while *C. concisus* was isolated from 6 cases and 4 controls. In contrast, MLPA detected Epsilonproteobacteria in 22.5% (39/173) of cases and 11.5% (19/165) of controls. *C. concisus* was the most common species detected by MLPA in both cases (23, 13.3%) and controls (14, 8.5%). *C. jejuni* was detected in 17 (9.8%) cases and 1 (0.61%) control. Fifty-four faecal extracts (16.0%) had discordant results between culture and MLPA. The majority (20 cases and 16 controls, 66.7%) were positive by MLPA and negative by culture, and 16 (10 cases and 6 controls, 29.6%) were positive by culture and negative by MLPA. The prevalence of *C. jejuni* was significantly higher in cases than controls using both culture ($p < 0.0001$, Odds Ratio [OR] = 9.5) and MLPA ($p = 0.00041$, OR = 17.9) and the difference in prevalence of *C. concisus* was not significant by either culture or MLPA. These results support *C. jejuni* as the most common Epsilonproteobacterial cause of childhood gastroenteritis in Belgium but the role of *C. concisus* remains unsubstantiated.

Could gastric microbiota be the cause of reactive gastropathy?

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Backgrounds: As the cause of reactive gastropathy (RG), bile, pancreatic secretions, alcohol, NSAIDs, and many chemicals and drugs have been suggested. RG associated with gastric microbiota has not been paid much attention. We have proposed a name of a novel bacterium *Okadaella gastrococcus (Og),* which co-exists with *H. pylori,* is an intracellular acid tolerant Gram-variable bacterium. The bacterium produces H_2S, various enzymes including DNase and CHO cell sensitive cytotoxin. The aim was to investigate if any microorganism including *Og* could be associated with RG.

Methods: The data of 22 patients (M:F 7:15, age: 21-80 years) who were found to suffer from *Og*-like bacteria positive RG (OGLB-RG) by histology (H&E, WSS, AYTB, Diff Quick stains) were used. They were free from NSAIDs, alcohol, smoking and renal dysfunction. The formalin-fixed, paraffin-embedded specimens from 5 patients were examined with *Og* and *H. pylori* immunohistochemistry. The specimens from 9 patients were examined under TEM. Culture of gastric biopsy specimens was attempted from 11 patients. Statistical analysis was performed by Fisher's Exact test.

Results: Gastric erosion, petechial hemorrhage/hematin (PH/H), duodenitis, and inflammation of squamo-columnar junction were found in 20 (90.91%, n=22), 14 (82.35%, n=17), 14 (82.35%, n= 17), 11 (100%, n= 11), respectively. Statistical analysis revealed the positive association of PH/H with duodenitis (p=0.001), and of chronic gastritis with post-*H. pylori* eradication (p=0.02) in OGLBP-RG. OGLB were found in the interstitial space and areas of intestinal metaplasia. Unidentified OGLB were cultured from two patients but subculture was not successful. *Propionibacterium acnes* was isolated from a Japanese male. Three types of OGLBP-RG classification are proposed from the present results.

Conclusion: Present findings support a possible involvement of bacteria in the development of the pathology. Type III RG with pre-neoplastic gastropathy warrants careful follow-up in the population who has a higher incidence of gastric cancer.

First description of *Campylobacter lanienae* from feces of organic and conventional pigs, in France

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In the frame of the CORE Organic II funded European project SafeOrganic, fecal samples of 58 conventional pigs and 56 organic pigs, originated from 31 organic herds and 31 conventional herds, were collected in a slaughterhouse in order to isolate *Campylobacter coli*. Direct streaking from feces and incubation at 37°C of the Karmali plates allowed the isolation of another *Campylobacter* species: *Campylobacter lanienae*.

Indeed, among the 381 typical Campylobacter colonies isolated, it was not possible to identify the species for 118 isolates with the Wang's multiplex-PCR. However, 85 of these isolates were confirmed *C. lanienae* by Maldi-Tof and by 16S rRNA PCR. With the two species, *coli* and *lanienae*, the occurrence of Campylobacter in pig was estimated to 87.9% (51/58) for conventional pigs and 96.5% (52/56) for organic pigs.

A total of 55 isolates of *C. lanienae* were tested for their resistance to 7 antibiotics. Only one was pansusceptible. Natural resistance of this species to Nalidixic acid was confirmed. Resistance to Tetracycline was significantly different between the two productions ($p <$ to 0.001) : 88 % of the conventional pig isolates were resistant against 14% of organic pig isolates. Moreover, 73% of the conventional pig isolates were multiresistant against 5% of organic pig isolates. The *C. lanienae* isolates were typed by PFGE using *Kpn*I and *Sma*I enzymes. The genetic diversity was very high, whatever the enzyme used (ID > to 0.98). No link between PFGE profile and isolate origin or antibiotic resistance pattern was evidenced.

This study allowed us to demonstrate for the first time in France that pigs may also carry in their feces a species rarely highlighted: *C. lanienae*. The lower level of antibiotic resistance and multiresistance of *C. lanienae* strains for organic pigs may be related to the restricted use of antibiotics in this production.

Investigation of the Function of T4SS and T6SS in *Campylobacter rectus*

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Campylobacter rectus is an anaerobic oral *Campylobacter* species associated with periodontal disease. It is found in 90% of adults with new or prolonged periodontitis. It has been recently linked to Barrett's esophagus and abscesses, both oral and extraoral. Additionally, *C. rectus* infections in pregnant women have been suggested to be associated with pre-term birth and low infant birth weight. As *C. rectus* is an oral pathogen, we are investigating possible virulence factors present in this organism.

Through genome sequencing, we have found genes for a type IV secretion system (T4SS) in several *C. rectus* strains and a type VI secretion system (T6SS) genes in all of our *C. rectus* strains. T4SS are used in the uptake and transport of DNA, but in some bacteria also act as a mode of protein delivery. We analyzed *C. rectus'*s ability to take up DNA by comparing the rate of uptake and transformation of several strains as well as a T4SS mutant Δ*virB9.*

T6SS have a variety of functions in different types of bacteria. It is a protein transport system that may be used as an antimicrobial weapon and a toxin delivery system to host organisms. We have generated a T6SS mutant, Δ*hcp,* and are investigating the function of T6SS in *C. rectus* through competition assays with other common oral bacteria found in the progression of periodontitis and gingivitis as well as the impact on its host through host cell response. By studying *C. rectus's* T4SS and T6SS we are aiming to gain a better understanding of its pathogenesis.

Updating the genomic taxonomy and epidemiology of *Campylobacter hyointestinalis*

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Although *Campylobacter jejuni* and *Campylobacter coli* are the most common causative agents of human campylobacterosis worldwide, many other species of Campylobacter have been associated with human gastrointestinal infection. Such species can be of great interest due to their alternative, sometimes acute, virulence when infecting humans and/or their high prevalence in the environment, domestic and/or wild animal species. *Campylobacter hyointestinalis* falls into these categories.

During our routine surveillance of Campylobacter in ruminant livestock, we identified *C. hyointestinalis* as commonly infecting farmed cattle, sheep and deer in New Zealand. To assess the relevance of our findings, we performed a systematic literature review. Originally described in 1983, *Campylobacter hyointestinalis* was classically identified as a causative agent of enteric disease in pigs many of which had proliferative ileitis. However, the most frequently reported source for *C. hyointestinalis* infection since its discovery has been the faeces of healthy cattle herds and humans with various presentations of gastroenteritis. Currently available genetic data distinguishes two subspecies of *C. hyointestinalis*; subspecies *hyointestinalis* which is most commonly associated with cattle, and subspecies *lawsonii* which is commonly found in pigs. However, epidemiological data that distinguishes between subspecies remains scarce, and subject to intense sampling bias.

Draft genome sequence data from our study were compared to all other publicly available *C. hyointestinalis* genomic data. This demonstrated that, similarly to *C. jejuni* and *C. coli*, the *C. hyointestinalis* genome is extremely plastic in its nature with recombination, horizontal gene transfer, gene acquisition and loss all being commonplace. We observed that such events can easily lead to misinterpretation of genetic similarity between species, and used recombination-stripping methodology to provide an accurate global phylogeny for *C. hyointestinalis* species based on available multi-locus sequencing data from PubMLST. Our analyses provide an up-to-date description of the global taxonomy and epidemiology of *C. hyointestinalis*.

Oxygen availability influences motility and biofilm formation of the emerging gastrointestinal pathogen, *Campylobacter concisus*

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Introduction: *Campylobacter concisus* is associated with gastroenteritis and inflammatory bowel diseases (IBD), but is also found in healthy subjects. Strain differences may explain virulence differentials, alternatively virulence may depend on changes in the gastrointestinal environment. Oxygen levels differ in the healthy and inflamed GI tract and oxygen has been found to regulate bacterial virulence. Motility and biofilm formation are necessary for bacterial colonization and to date have been poorly characterized in *C. concisus*. In this study we characterize the effect of varying oxygen availability on motility and biofilm formation of *C. concisus* isolates from healthy subjects and patients with gastrointestinal disease.

Methods: Biofilm formation and motility of *C. concisus* isolated from saliva, gut mucosal biopsies and feces of 39 individuals with either IBD (n=23), gastroenteritis (n=8), or healthy subjects (n=13) were quantified after 72 hours of growth under microaerophilic or anaerobic conditions. Biofilm formation was measured *via* crystal violet staining and motility by motility zone diameters on soft agar plates.

Results: Motility and biofilm formation were not related to GI disease status. However, motility differed significantly when strains were cultured under anaerobic or microaerophilic growth conditions **($p<0.001$),** with strong increase in motility under microaerophilic conditions. A similar phenomenon was observed for biofilm formation, but only in a subset of isolates. Oral isolates exhibited significantly increased biofilm formation compared with fecal isolates **($p<0.03$),** and exhibited a strong negative correlation between motility and biofilm formation **($R2 = -0.7$; $p=0.01$).**

Conclusion: *C. concisus* motility and biofilm formation was not related to GI disease status. Motility was strongly related to oxygen availability, this was also observed for biofilm formation, but only in a subset of isolates, suggesting that oxygen influences the biofilm and motility capacity of *C. concisus* and that the inflamed gut may dictate the physiology of this opportunistic pathogen.

Investigating the virulence characteristics of the emerging pathogen *Campylobacter concisus*

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C. concisus is a resident of the human oral which has been linked to gingivitis, periodontitis, gastroenteritis and inflammatory bowel disease (IBD) symptoms. However, the pathogenic role of this bacterium in these conditions remains unclear. Our research aims to investigate the virulence characteristics of *C. concisus* strains isolated from healthy children and adults and from clinical samples of IBD patients. The expression of several putative virulence-related genes in oral and intestinal strains was investigated under different environmental conditions. This included *flaA*, *flaB*, *flaC* flagellin genes; the *cjaA, cjaC* of the ABC transport system; the heat shock protein, *dnaJ*; and the tight junction toxin gene, *zot*. Other virulence characteristics including adhesion, invasion, motility, biofilm formation and the role of *luxS* in virulence were also investigated. Our data indicated that the oral cavity of healthy children can be colonised with virulent *C. concisus* strains including the most virulent strain in our collection which was isolated from a six year old healthy child. Furthermore, a *luxS* mutant that was created in this strain showed a significant reduction in virulence when compared to the parental strain Therefore it remains unclear whether the virulence characteristics of the colonising *C. concisus* strains or the immune status of the individual are the main factors in initiating the infection.

Multi locus sequence typing and detection of virulence genes in *Campylobacter concisus* isolates from Denmark, using whole genome sequence data

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Introduction: *Campylobacter concisus* has been linked to various enteric diseases such as inflammatory bowel disease (IBD), yet has also been isolated from the intestine of healthy controls (HC). It has been proposed that different *C. concisus* strains, or virulence-associated genes such as *zot* and exotoxin 9/DnaI, may be involved in the inflammatory process, possibly explaining differences in the pathogenic potential of *C. concisus* strains from different clinical presentations.

Materials and methods: Genomes of 104 clinical *C. concisus* isolates from different sites (saliva n=14, mucosal biopsies n=70, faeces n=20) in 27 individuals (IBD=18, HC=7, gastroenteritis=2) were sequenced. The sequences of seven housekeeping genes were subsequently aligned and compared using the Bacterial Isolate Genome Sequence database (BIGSdb), and phylogenetic trees were generated using the MEGA7 tool. The DNA sequence for the *zot* and exotoxin 9/DnaI genes were obtained from the NCBI database and aligned by BLAST in BIGSdb. Nucleotide sequences and amino acid compositions were visualized with the BioEdit software.

Results: Multi locus sequence typing (MLST) divided the isolates into two main genomospecies (GS). While there was a predominance of oral isolates in GSI and mucosal isolates in GSII ($p<0.0001$), there was no association to clinical presentation ($p=1.0$). In total, eight isolates had the *zot* gene only, 50 isolates had the exotoxin 9/DnaI gene only, and there was no association to clinical presentation. Nucleotide sequences of the *zot* genes did not reveal polymorphisms specific to disease groups. Nine isolates had both virulence genes, and while these were all from IBD patients, this did not reach statistical significance.

Conclusions: The vast heterogeneity of *C. concisus* isolates was confirmed. Neither MLST, nor the prevalence of putative virulence genes *zot* or exotoxin 9/DnaI correlated to clinical presentation. Analysis of complete whole-genome sequence data is pending and this may highlight possible correlations to intestinal disease.

Prevalence of *Campylobacter concisus* and *C. ureolyticus* in travelers' diarrhea cases and asymptomatic controls

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Introduction: *Campylobacter concisus* and *C. ureolyticus* have been described as emerging pathogens associated with acute gastroenteritis, Inflammatory Bowel Disease and Crohn's Disease. This study aims to investigate the prevalence and significance of both species in stool from travelers with diarrhea and asymptomatic controls.

Material and Methods: A total of 826 stool samples from studies of travelers' diarrhea etiology conducted in Thailand (163 cases and 75 controls) and Nepal (426 cases and 162 controls) were assayed using PCR to detect *Campylobacter* Genus (16S *rRNA*) and *C. concisus* and *C. ureolyticus* which were differentiated using primers for species-specific chaperonin genes (*Cpn60*).

Results: *C. concisus* was detected in 12/163 (7.4%) cases and in no controls (*P*=0.0099) in stool samples collected in Thailand. For Nepal stool samples, it was detected in 54/426 (12.7%) cases and 7/162 (4.3%) controls (*P*=0.0023). *C. ureolyticus* was not detected in any of the cases, but was identified in one control stool sample from Thailand and was detected in 2/426 (0.5%) cases and 3/162 (1.9%) controls in stool samples from Nepal. Among the 66 *C. concisus* isolates from cases and 7 isolates from controls, 34.8% and 42.8% were detected as a single pathogen, respectively.

Conclusion: *C. concisus* was detected significantly more in cases as compared to asymptomatic controls highlighting the importance of *C. concisus* as an emerging or reemerging enteric pathogen. Further studies are needed to elucidate potential pathogenic mechanisms of *C. concicus* as they relate to gastroenteritis development and pathology.

Frequency of *Campylobacter ureolyticus* in stools of patients with gastrointestinal disorders in a central Bohemian regional hospital

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Introduction & Objectives: *Campylobacter ureolyticus*, an urease-positive, hydrogen-requiring *Campylobacter* species is considered an emerging gastrointestinal pathogen. However, its etiological role is not yet clarified, partially due to a large intra-species heterogeneity. The aim of our one-year, systematic study was to determine the frequency of *C. ureolyticus* in stools of patients with gastrointestinal disorders using a culture-based method. Furthermore, co-infections, seasonal, age and sex distribution were assessed.

Material & Methods: Between April 2015 and March 2016, consecutive rectal swabs from 2234 patients were routinely tested for common gastrointestinal pathogens in a regional hospital. In addition, all these samples were examined for *C. ureolyticus* by culture on Skirrow selective medium (Oxoid) under microaerobic conditions. Suspect *C. ureolyticus* colonies were identified to species level using commercial biochemical tests (ANAEROtest 23, Lachema) and subsequently confirmed by MALDI TOF MS (Biovendor). A species-specific PCR was used for verification in a subset of randomly selected *C. ureolyticus* isolates.

Results: *C. ureolyticus* was detected in 25% of patients, followed by *C. jejuni/C. coli* (9%). In 5.2% of patients *C. ureolyticus* occurred in a mixed infection, mostly with other *Campylobacter* spp. In 82% of the positive patients the isolation of *C. ureolyticus* correlated with its putative etiological involvement (> 2/3 of such patients had gastroenteritis, the rest other gastrointestinal manifestations). Seven percent shed *C. ureolyticus* asymptomatically. The frequency of *C. ureolyticus* was higher in females and the highest in children < 9 years. The seasonal distribution differed from *C. jejuni/C. coli*.

Conclusions: Using a selective culture for the detection of *Campylobacter* spp., the detection rate of *C.ureolyticus* was high. It replaced *C. jejuni*/*C. coli* as the most frequent *Campylobacter* spp. in stools of patients with gastrointestinal disorders, particularly with gastroenteritis. Our data support the etiological role of *C. ureolyticus* in human gastrointestinal diseases. MALDI TOF is a an accurate and rapid tool for identification of emerging *Campylobacter* spp. in clinical samples.

Past, Present and Future
of the Taxonomy of the Genus *Arcobacter*

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The genus *Arcobacter* embrace species isolated worldwide from animals, humans, wastewater, freshwater, marine ecosystems and several food products. Since the genus descriptions in 1991-92 with 4 species, the number remained constant up to 2005 when it increased to 6 species, but it raised exponentially with yearly new descriptions from the 8 species of 2009 to 25 species in 2016. The aim of this presentation is to show the key elements for such fast evolution and to underline how we foresee in the genomic era the future taxonomy of this genus.

Phenotypic characterization, 16S rRNA gene sequences and DNA–DNA hybridization (DDH) were the classical methods used for delineating species that evolved to the use of sequences of housekeeping genes (rpoB etc) to perform a Multilocus Phylogenetic Analysis (MLPA) with the concatenated sequences. More recently the average nucleotide identity (ANI) and the in silico DDH that compare the genomes of the potential new species with the nearest known species are the methods of reference.

Of the 25 species, 11 (42.3%) were discovered by our group using the 16S rRNA-RFLP identification method we developed and by the routine used of the rpoB sequences. The latter together with the MLPA due to its higher resolution showed to be more accurate than the 16S rRNA gene for the delineation the species. The genomes of some potential new species showed ANI values >96% and isDDH results >70% with their closes relatives determined on the basis of MLPA.

The genomes of all the type strains of the accepted species should be obtained. The MLPA, ANI and isDDH are excellent tools for delineating species. On the basis of phylogenetic distances and other data some of species of the genus *Arcobacter* could be grouped in the near future into a new and independent genus.

Analysis of 28 *Arcobacter* genomes belonging to different species

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The genus *Arcobacter* belongs to the family *Campylobacteraceae* and can be differentiated from *Campylobacter* due to their ability to growth at low temperatures and in aerobiosis. Since the description of the genus in 1991, a total of 25 species have been described, most of them in the last decade. Eleven genomes of the known species are available at the NCBI together with four unlabeled genomes. In our lab four genomes of other known species and nine new candidates were sequenced. The aim of the present study was to analyze the features of these genomes.
Annotation was carried out using Prodigal and comparison of the proteomes of the 28 genomes was performed with CMG-biotools. Core and pan genomes were extracted and a core-genome tree was constructed. Virulence and resistance genes were searched for. Average Nucleotide Identity (ANI) and *in silico* DNA-DNA hybridization (*is*DDH) was calculated for all the genomes to verify their identity.
The G+C content of the genus ranged from 26.4% to 34.9% and the genome size was between 1.7 and 3.6 Mb. The genomes were identified as different species on the basis of ANI and *is*DDH results below 96% and 70%, respectively. Only one copy of the 16 rRNA gene was found in the analyzed genomes. Number of tRNA ranged from 32 to 79 and CDS ranged from 1801 to 3636. Core genome was composed for 910 genes while 12,328 corresponded to accessory genes. Virulence genes *dnaJ* and *ciaB* were found in all the tested species while *cadF*, *cj1349*, *irgA*, *hecA*, *hecB* and *mviN* were found in different percentages in the studied genomes, independently of their origin. Resistance genes detected were: β-lactamic antibiotics, tetraciclines, nalidixic acid, macrolides and chloramphenicol.
Genomic data clarified the taxonomy and ecological role of these bacteria.

Intestinal expression of genes encoding inflammatory mediators and gelatinases during *Arcobacter butzleri* infection of secondary IL-10 deficient mice

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Backgound: We have previously shown that *Arcobacter butzleri* induce intestinal, extra-intestinal and systemic immune responses in perorally infected secondary abiotic IL-10-/- mice in a strain-dependent fashion. Here we present a comprehensive survey of small and large intestinal expression profiles of inflammatory and regulatory mediators as well as of the matrix-degrading gelatinases MMP-2 and MMP-9 following murine *Arcobacter butzleri* infection.

Methodology / Results: Secondary abiotic IL-10-/- mice were generated by broad-spectrum antibiotic treatment and infected with *A. butzleri* strains CCUG 30485 or C1 of human and chicken origin, respectively. At day 6 following *A. butzleri* infection, mucin-2 mRNA, an integral part of the intestinal mucus layer, was down-regulated in the colon, whereas TNF and IL-23p19 mRNA were up-regulated in the ileum. Furthermore, IFN-gamma, IL-17A, IL-1beta, and IL-22 mRNA were up-regulated in both, colonic and ileal *ex vivo* biopsies at day 6 post strain CCUG 30485 infection. These changes were accompanied by down-regulated colonic MMP-9 levels, whereas both, MMP-2 and MMP-9 mRNA were up-regulated in the ileum.

Conclusion: Our data indicate that *A. butzleri* infection induces changes in the expression of genes involved in pro-inflammatory and regulatory immune responses as well as in tissue degradation.

Arcobacter butzleri induce colonic, extra-intestinal and systemic inflammatory responses in secondary abiotic IL-10 deficient mice

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Introduction: The immunopathological impact of human *Arcobacter* infections is under current debate. Episodes of gastroenteritis with abdominal pain and acute or prolonged watery diarrhea were reported for *A. butzleri* infected patients. Whereas adhesive, invasive and cytotoxic capacities have been described for *A. butzleri in vitro*, only limited information is available about the immunopathogenic potential and mechanisms of infection *in vivo*.

Methodology / Results: Secondary abiotic IL-10-/- mice were generated by broad-spectrum antibiotic treatment and perorally infected with the *A. butzleri* reference strain CCUG 30485, isolated from a diseased patient, or with the C1 strain derived from fresh chicken meat. Bacterial colonization capacities, clinical conditions, intestinal, extra-intestinal and systemic immune responses were monitored at day six and 16 postinfection (p.i.). Despite stable intestinal *A. butzleri* colonization at high loads, secondary abiotic IL-10-/- mice were virtually uncompromized and did not display any overt symptoms at either time point. Notably, *A. butzleri* infection induced colonic apoptosis, which was paralleled by increased abundance of proliferating cells. Furthermore *A. butzleri* infection caused a significant increase of distinct immune cell populations such as T and B cells, regulatory T cells, macrophages and monocytes in the colon which was accompanied by elevated colonic TNF, IFN-gamma, nitric oxide (NO), IL-6, IL-12p70 and MCP-1 concentrations. Strinkingly, *A. butzleri* induced extra-intestinal and systemic immune responses as indicated by higher NO concentrations in kidney and increased TNF, IFN-gamma, IL-12p70 and IL-6 levels in serum samples of infected as compared to naïve mice. Overall, inflammatory responses could be observed earlier in the course of infection by the CCUG 30485 as compared to the C1 strain.

Conclusion / Significance: Peroral *A. butzleri* infection induced not only intestinal but also extra-intestinal and systemic immune responses in secondary abiotic IL-10-/- mice. These findings point towards an important immunopathogenic potential of *A. butzleri* in vertebrate hosts.

Survey of small intestinal and systemic immune responses following murine *Arcobacter butzleri* infection

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Background: *Arcobacter butzleri* has been described as causative agent for sporadic cases of human gastroenteritis with abdominal pain and acute or prolonged watery diarrhea. *In vitro* studies revealed distinct adhesive, invasive and cytotoxic properties of *A. butzleri*. Information about the underlying immunopathological mechanisms of infection *in vivo*, however, are scarce. The aim of this study was to investigate the immunopathological properties of two different *A. butzleri* strains in a well-established murine infection model.

Methodology / Results: Secondary abiotic IL-10-/- mice, in which the intestinal microbiota was depleted following broad-spectrum antibiotic treatment, were perorally infected with two different *A. butzleri* strains isolated from a diseased patient (CCUG 30485) or fresh chicken meat (C1), respectively. Even though bacteria of either strain could stably colonize the intestinal tract at days 6 and 16 postinfection, mice did not exert infection-induced symptoms such as diarrhea or wasting. In small intestines of infected mice, however, increased numbers of apoptotic cells could be detected at day 16, but not day 6 following infection with either strain. A strain-dependent influx of distinct immune cell populations such as T- and B-cells as well as of regulatory T-cells could be observed upon *A. butzleri* infection which was accompanied by increased small intestinal concentrations of pro-inflammatory cytokines including TNF, IFN-gamma, MCP-1 and IL-6. Remarkably, inflammatory responses following *A. butzleri* infection were not restricted to the intestinal tract, given that the CCUG 30485 strain induced systemic immune responses as indicated by increased IFN-gamma concentrations in spleens at day 6, but not day 16 following infection.

Conclusion: Upon peroral infection *A. butzleri* stably colonized the intestinal tract of secondary abiotic IL-10-/- mice. The dynamics of distinct local and systemic inflammatory responses could be observed in a strain-dependent fashion pointing towards an immunopathogenic potential of *A. butzleri in vivo*. These results indicate that secondary abiotic IL-10-/- mice are well suited to further investigate the molecular mechanisms underlying arcobacteriosis *in vivo*.

Toll-like-Receptor-4 is essential for *Arcobacter butzleri* induced colonic and systemic immune responses in secondary abiotic IL-10-/- mice

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Introduction: *Arcobacter butzleri* have been shown to cause sporadic cases of human gastroenteritis with abdominal pain and acute or prolonged diarrhea. Information about the underlying immunopathological mechanisms of infection, however, is limited. For the first time we investigated the role of Toll-like-Receptor (TLR) -4, the main innate receptor for lipopolysaccharide and lipooligosaccharide of Gram-negative bacteria, in murine *Arcobacter* infection.

Materials and methods: Secondary abiotic TLR-4 IL-10 double deficient (TLR-4-/- IL-10-/-) and IL-10-/- control mice were generated by antibiotic treatment and perorally infected with *A. butzleri*.

Results: Until day 16 postinfection TLR-4-/- IL-10-/- and IL-10-/- control mice were stably colonized by *A. butzleri*. Infected IL-10-/- mice lacking TLR-4 displayed less pronounced colonic apoptosis that was accompanied by lower numbers of innate and adaptive immune cells within the colonic mucosa and lamina propria as compared to IL-10-/- controls. Furthermore, large intestinal pro-inflammatory mediators including nitric oxide, TNF, IL-6 and MCP-1 and, remarkably, of systemic pro-inflammatory cytokines such as IFN-γ and IL-12p70 were lower in *A. butzleri* infected TLR-4-/- IL-10-/- vs IL-10-/- mice.

Conclusion: TLR-4 is involved in mediating *Arcobacter* infection *in vivo*. Further studies are needed to investigate the molecular mechanisms underlying arcobacteriosis in more detail.

Toll-like Receptor-4 dependent small intestinal immune responses following murine *Arcobacter butzleri* infection

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Introduction: Sporadic cases of gastroenteritis have been attributed to *Arcobacter butzleri*-infection, but information about the underlying immunopathological mechanisms is scarce. We have recently shown that experimental *A. butzleri*-infection induces intestinal, extraintestinal and systemic immune responses in secondary abiotic IL-10-/- mice. The aim of the present study was to investigate the immunopathological role of Toll-like Receptor-4, the receptor for lipopolysaccharide and lipooligosaccharide of Gram-negative bacteria, during murine *A. butzleri*-infection.

Methodology / Results: To address this, secondary abiotic IL-10-/- mice lacking TLR-4 were generated by broad-spectrum antibiotic treatment and perorally infected with two different *A. butzleri* strains isolated from a patient (CCUG 30485) or fresh chicken meat (C1), respectively. Bacteria of either strain stably colonized the ilea of mice irrespective of their genotype at days 6 and 16 postinfection. As compared to IL-10-/- control animals, TLR-4-/- IL-10-/- mice were protected from *A. butzleri* -induced ileal apoptosis, from ileal influx of adaptive immune cells including T-lymphocytes, regulatory T-cells and B-lymphocytes, and from increased ileal IFN-gamma secretion.

Conclusion: Given that TLR-4-signaling is essential for *A. butzleri*-induced intestinal inflammation, we conclude that bacterial lipopolysaccharide or lipopolysaccharide compounds aggravate intestinal inflammation and may thus represent major virulence factors of *Arcobacter*. Future studies need to further unravel the molecular mechanisms of TLR-4-mediated *A. butzleri*-host interactions.

Toll-like Receptor-4 dependent intestinal gene expression during *Arcobacter butzleri* infection of secondary abiotic IL-10 deficient mice

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Introduction: We have previously shown that *Arcobacter butzleri* infection induces Toll-like Receptor (TLR) -4 dependent immune responses in perorally infected secondary abiotic IL-10-/- mice.

Methodology / Results: Here we analyzed TLR-4 dependent expression of genes encoding inflammatory mediators and matrix-degrading gelatinases MMP-2 and -9 in the small and large intestines of secondary abiotic TLR-4 deficient IL-10-/- mice that were perorally infected with *A. butzleri* strains CCUG 30485 or C1, of human and chicken origin, respectively. At day 6 following *A. butzleri* infection, colonic mucin-2 mRNA, as integral part of the intestinal mucus layer, was down-regulated in the colon, but not ileum, of IL-10-/-, but not TLR-4-/- IL-10-/- mice. CCUG 30485 strain infected TLR-4-deficient IL-10-/- mice displayed less distinctly up-regulated IFN-gamma, IL-17A and IL-1beta mRNA levels in ileum and colon, which was also true for colonic IL-22. These changes were accompanied by up-regulated colonic MMP-2 and ileal MMP-9 mRNA exclusively in IL-10-/- mice.

Conclusion: TLR-4 is essentially involved in *A. butzleri* mediated modulation of gene expression in the intestines of secondary abiotic IL-10-/- mice.

Fitness cost of ciprofloxacin resistance in *Arcobacter butzleri*

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Antibiotic resistance has been increasing in microbial populations, with bacteria suffering fitness cost. *A. butzleri* resistance to several classes of antibiotics, namely fluoroquinolones, has been described for strains isolated from diverse sources; however, there is still a lack of information regarding fitness of *A. butzleri* resistant strains. The aim of this work was to understand the impact of ciprofloxacin resistance on the fitness of *A. butzleri* and to evaluate how it could influence its survival and persistence.

A. butzleri was subjected to *in vitro* induction experiment conducted using susceptible strains and ciprofloxacin as a selecting agent to obtain ciprofloxacin-resistant mutants, starting with exposure to sub-inhibitory antibiotic concentrations and increasing until growth cessation. Ciprofloxacin-susceptible parent and resistant strains were evaluated regarding the minimum inhibitory concentration (MIC) of ciprofloxacin and the quinolone resistance determining region (QRDR) of *gyrA* gene of all the strains was sequenced. Changes in the fitness between the susceptible parent strains and ciprofloxacin-resistant mutants were examined through growth rate evaluation, pairwise competition and survival ability to stress factors.

The repeated exposure to ciprofloxacin resulted in a decrease of susceptibility to ciprofloxacin in all mutant strains, involving different mutations in the QRDR of GyrA: single mutations (Thr-85-Ile or Asp-89-Tyr) or double mutation (Thr-85-Ile and Asp-89-Tyr). The Asp-89-Tyr amino acid substitution conferred a MIC of 64 µg/mL, however with the single mutation Thr-85-Ile or the double mutation leading to higher ciprofloxacin resistance (>256 µg/mL). Although there were no statistical significant differences in doubling time and survival to heat, freeze and oxidative stresses, the relative fitness differed from pair to pair. These results demonstrate that mutations conferring ciprofloxacin resistance may not affect *A. butzleri* ability to tolerate and persist under stress conditions or that compensatory mechanisms may amend the cost assigned by resistance, nonetheless the fitness cost is strain dependent.

Detection of *Arcobacter* spp.
along the intestinal tract of broiler chickens

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Background and objectives: *Arcobacter* spp. is regularly detected in chicken meat. The intestinal content of the chickens is assumed as the source of contamination during the slaughtering process. However, data on the occurrence and quantitative load of *Arcobacter* spp. along the complete intestinal tract of chicken are still scarce. Therefore, we examined the intestinal content of the duodenum, jejunum, caeca and colon of broiler chickens individually to test for the presence and the concentration of *Arcobacter* spp.

Materials and methods: Intestinal tracts of 25 broiler chickens from 5 different flocks were collected and intestinal content of the duodenum, jejunum, caeca and colon was extracted. Of each sample, 1 g was examined for the presence and quantitative load of *Arcobacter* spp. by selective enrichment. Suspected *Arcobacter* colonies were verified by mPCR and rpoB sequencing.

Results: In 44% (11/25) of the duodenal, 64% (16/25) of the jejunal, 8% (2/25) of the caecal and 92% (23/25) of the colonic samples *A. butzleri* were detected. The highest *Arcobacter* load was determined in the colonic content (23 MPN/g), followed by duodenum and jejunum (0.023 – 0.23 MPN/g), respectively.

Conclusion: Our data support the hypothesis that the intestinal tract has to be considered a source of entry for *Arcobacter* spp. into the poultry slaughterhouse, thereby enabling contamination and cross-contamination of the chicken meat. In contrast to *Campylobacter*, the highest numbers of *Arcobacter* spp. were detected in the colon, whilst the caeca showed the lowest *Arcobacter* concentration. We were also able to detect *Arcobacter* spp. in the small intestine, however, with lower bacterial numbers compared to colon. Therefore we recommend to use colonic content when testing for the presence of *Arcobacter* spp. in chicken at slaughter.

Comparison between the incidence of *Arcobacter* from water and shellfish using the MPN method and two culture methods in parallel

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Arcobacter spp. can be pathogenic to humans and animals. Few studies have enumerated *Arcobacter* in shellfish and water. Therefore we studied the concentrations of *Arcobacter* and *Escherichia coli* in water and shellfish collected in Alfacs Bay (AB) and in shellfish exposed to a fecally contaminated freshwater channel (CCh).
The objective is to evaluate if the MPN *Arcobacter* protocol, employed in previous studies, is useful for determining the presence of these bacteria in shellfish and water samples with different loads of fecal contamination.
We studied 44 samples (21 water and 23 shellfish) from AB and 31 from CCh (12 water and 19 shellfish). The *Arcobacter* 5 tubes MPN method contained 5.5 ml or g of the original sample. The MPN results were compared with data from two culture methods where 1g of shellfish and 200 ml of water were analyzed: A) enrichment in *Arcobacter* CAT broth + passive filtration on blood agar and B) same broth with 2.5% NaCl and filtration on marine agar. All samples were analyzed for *E. coli* (MPN ISO/TS 16649-3:2005).
From AB, 14 (31.8%) samples were simultaneously positive by MPN and both culture approaches (5 samples by method A and 19 by method B), 19 (43.2%) were only positive by culture and 2 (4.5%) only by MPN. Of the 57.6% (19/33) MPN *Arcobacter* false negative samples, 12 (63.2%) corresponded to water samples and 7 to shellfish (36.8%). All 31 samples from CCh were positive for *Arcobacter* with all methods.
The underestimation by the *Arcobacter* MPN occurred only at the AB harvesting area (with *E. coli* levels <230 MPN·100g-1) where probably the amount of sample tested was too low. The original MPN was useful for heavily fecally contaminated samples like those of CCh (mean MPN values of *E. coli* and *Arcobacter* were 5.6x104 and 5.0x105, respectively).

The Prevalence and Molecular Typing and Antimicrobial Resistance of *Arcobacter* spp. Isolated from Raw Chicken Meat in Beijing

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The *Arcobacter* genus belonging to the family Campylobacteraceae, is associated with human and animal illness. We investigated the prevalence of the *Arcobacter* spp. from retail chicken meat in Beijing. The genotype and antimicrobial susceptibility profiles of these isolates were analyzed in this study.

A total of 60 retail chicken meat samples were randomly purchased from six retail outlets in Beijing. Enhanced membrane filter technique was used to isolate the *Arcobacter* spp.. 43 of 60 samples (71.67%) were positive. Among the 43 positive samples，125 *Arcobacter* isolates were obtained which include 114 *A. cryaerophilus,* 10 *A. butzleri* and 1 *A. skirrowii*. Nine of the 60 samples (15%) were found to be mix contaminated with more than one *Arcobacter* species.

Totally, 53 isolates (43 *A. cryaerophilus,* 9 *A. butzleri,* 1 *A. shirrowii)* were analyzed by multi-locus typing and the *minimum inhibit*ory concentrations for six drugs were measured with agar dilution method. The resistance break points were used according to the standards used in the NARMS for *Enterobacteriaceae* in USA. 53 isolates were classified in 43 sequence types (STs) including 33STs in 43 *A. cryaerophilus*, 9 STs in 9 *A. butzleri* and one ST in one *A. shirrowii* isolate. All of the 43 STs were unreported before. A total of 231 alleles were identified across all seven loci, and among them, 145 new alleles were novel identified. For *A. butzleri*, no gentamicin- and tetracycline-resistant isolates were found among 9 strains and 78% strains were chloramphenicol resistance. For *A. cryaerophilus,* 56% strains were ciprofloxacin resistance. The proportion of multi-resistant *A. butzleri and A. cryaerophilus* was 22% and 16.9%, respectively.

Prevalence of *Arcobacter* spp. in retail seafood in Germany

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Background: *Arcobacter* species are considered emerging zoonotic pathogens which could provoke human gastroenteritis. They were already isolated from a wide range of habitats and hosts worldwide. However, information about the prevalence of *Arcobacter* in seafood products is still scarce.

Objective: The aim of this study was to investigate the prevalence of *Arcobacter* spp. in retail seafood like shellfish (mussels and clams), shrimps and cephalopods (squids and octopus) in Germany.

Methods: *Arcobacter* spp. were recovered and isolated by cultural methods using a selective enrichment step followed by a filter method. By mPCR and rpoB sequencing *Arcobacter* was verified at genus and species level.

Results: *Arcobacter* spp. were isolated from 55 out of 318 seafood samples. For cephalopods 26 % (28/106), shellfish 17 % (18/106) and shrimps 8.5 % (9/106) of the samples contained *Arcobacter* spp. Among all the isolates, 53 % belong to the species *A. butzleri* (33/62), followed by 14.5 % *A. venerupis* (9/62), 13 % *A. cryaerophilus* (8/62) and 11 % *A. aquimarinus* (7/62), while *A. skirrowii* and *A. thereius* were only detected once, respectively. Besides, three isolates could not be determined to species level by rpoB gene and 16S rRNA sequencing.

Conclusion: In this study, the prevalence of *Arcobacter* spp. in seafood samples was 17 %, which is in accordance with other studies of seafood products in Spain, Chile and India. To our knowledge, this is the first report of *Arcobacter* in cephalopods, in which *Arcobacter* could be detected with a rather high prevalence. These data support the potential risk of *Arcobacter* contamination and transmission to humans by consumption of retail seafood.

Prevalence, genetic diversity and resistance to antibiotics of *Arcobacter* spp. from retail food from Portugal

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Arcobacter genus belongs to *Campylobacteraceae* family, at the moment comprises 27 recognized species, some of which related with animal and human diseases. *Arcobacter*, mainly *A. butzleri*, is considered emerging foodborne pathogen presenting a risk to human health and a threat to food safety. In this work a total of 114 retail food samples from several retail markets and supermarkets were collected and analysed for the presence of *Arcobacter* spp.. The suspected colonies were identified by multiplex PCR and genotyped by ERIC-PCR, followed by antibiotic susceptibility testing to nine antibiotics by agar dilution. The results showed a high prevalence of *Arcobacter* spp. (60.5%), obtained by both molecular and culture detection; the highest frequency of detection observed was for poultry meat (92.0%), followed by fish (68.0%), ready-to-eat packaged vegetables (47.6%), pork meat (45.8%) and beef meat (42.1%). The most commonly isolated species were *Arcobacter butzleri* (58.5%) followed by *Arcobacter cryaerophilus* (35.8%) and *Arcobacter skirrowii* (5.7%). The genotyping of these isolates revealed a high genetic diversity; a possible cross-contamination through the finding of similar genotypes from different categories of food samples collected at the same supermarket was however detected. Concerning antibiotic resistance, even if all 105 tested isolates were shown to be susceptible to gentamicin, high rates of resistance were found to ampicillin (39.0%), tetracycline (90.5%), nalidixic acid (91.4%), cefotaxime (91.4%) and chloramphenicol (64.8%). Also, resistant isolates were observed for erythromycin (6.7%), ciprofloxacin (21.0%) and levofloxacin (18.1%); 85.7% of all isolates presented multidrug resistance. This study highlights the wide distribution of *Arcobacter* spp. in the food products analyzed and high rates of resistance to a broad spectrum of antibiotics. It is contribution to further acknowledge the *Arcobacter*-food contamination as a potential health hazard.

Isolation of *Okadaella gastrococcus II* sp. Nov.
from a *Helicobacter pylori* positive Japanese patient with dyspepsia

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Background: *Okadaella gastrococcus* (ATCC BAA-2258,NBRC 107862) is the name proposed for facultative anaerobic, motile and gram-stain-negative coccoid bacterium isolated from a human stomach with following biochemical characteristics; urease-, catalase-, oxidase-, PYR-negative, and arginine aminopeptidase-, alanine arylamidase-, glutamic acid decarboxylase-, DNase-, H_2S gas-positive. Intracellular presence of *O. gastrococcus (Og)* has been demonstrated in the classic gastric carcinogenic cascades and the subjects with *H. pylori* infection. The aim of this study was to examine if *Og* could be isolated from *H. pylori* positive patients with dyspepsia and the molecular study of an unidentified isolated microorganism by 16S rRNA and phylogenic analysis.

Methods: A 28 years old Japanese male, complaining of dyspeptic symptoms underwent an esophagogastroduodenoscopy. Cultures were attempted from gastric aspirate and biopsy specimens under microaerophilic and anaerobic conditions at 37°C for 3~14 days. Gram-stain-negative alpha-haemolytic motile coccoid isolates were subcultured further and biochemical tests were performed. The microorganism was subjected to 16S rRNA gene sequencing analysis and phylogenic tree reconstruction at TechnoSuruga laboratory. H&E, Diff-Quick and WSS stains were used in the histology examination.

Results: Histological examination confirmed the intracellular presence of *Og*-like organisms in the background of *H. pylori* positive active chronic gastritis. Unidentified bacteria with previously known biochemical and phenotypic features of *Og* were isolated successfully. The isolate was partially vancomycin sensitive. The results of 1500 b.p.16S rRNA gene sequencing analysis was 99.0% closely related with *S. parasanguinis*. The isolate had a distinctively independent branch of the phylogenic tree in the family of *Streptococcaceae* and formed clusters with *Og*.

Conclusions: *Og* co-exists with *H. pylori*. Phenotypic and genotypic features are adequate to distinguish *Og* from other microorganisms. A novel species within the genus *Okadaella* is proposed: *Okadaella gastrococcus II, sp. nov.* (NBRC 112295, GenBank K240440). DNA-DNA hybridization to endorse the species rank for the lineage is preferable.

Gram stain reactions of *Okadaella gastrococcus* (*Og*):
Gram stain negative to variable microorganism

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Background: *Og* is acid tolerant, CHO cell sensitive cytotoxin producing, facultative anaerobic, motile coccoid bacteria with unipolar hairy flagella and peritrichous fimbriae with terminal discs. *Og* can be isolated from a *H. pylori* positive pre-neoplastic human stomach. They have following biochemical characteristics; urease-, catalase-, oxidase-, PYR-negative, and arginine aminopeptidase-, alanine arylamidase-, leucine arylamidase-, alpha and beta galactosidase-, alkaline phosphatase-, alanyl-phenylalanyl-proline arylamidase-, glutamic acid decarboxylase-, DNase-, H_2S gas-positive. *Og* belongs to a family of Gram-positive bacteria *Streptococcaceae* but Gram stain reaction is ambiguous.

Objectives: To examine Gram stain reactions of three different *Og* strains.

Methods: *Og* (ATCC BAA-2258) and other two newly isolated strains (called here as S1, S2 and S3, respectively) were cultured at 37 ℃ under anaerobic conditions for 24 h to 72 h on chocolate agar, 5 % horse blood agar (HBA), 5 % HBA with brain heart infusion (BHI) plates, 5 % HB in BHI and BHI broths. Gram stain method (crystal violet, iodine, 95 % ethanol wash and safranin) and Favor method were used. *E. coli* and *Streptococcus pneumoniae* were used as the control of Gram-negative and Gram-positive bacteria, respectively.

Results: Gram stain reaction was unable to determine when the organisms were cultured with 5 % HB in BHI broth. S1 after 24 h incubation in BHI broth and all other strains immersed for 30 seconds in crystal violet were found to be more than 90 % Gram stain negative. All strains immersed for 1 minute in crystal violet showed Gram stain variable reactions. After 48 h incubation in BHI, some Gram positive chain forms were seen. Favor tests on S1, S2, and S3 showed Gram stain variable reactions.

Conclusion: *Og* showed Gram stain negative to variable reactions. Phenotypic, chemical and molecular cell wall structure studies are needed to clarify the Gram type of *Og*.

Whole-genome sequencing identifies three novel *Helicobacter* species isolated from gastric mucosa of red foxes

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Gastric helicobacters have been observed in stomachs in various species of carnivores. However, due to difficult cultivation and fragility of these bacteria, successful isolation attempts have only been reported for gastric material from dogs and cats or from captive wild carnivores, from which the samples are easier to obtain and more likely to be appropriate/fresh enough than in free-living wild carnivores. We successfully isolated seven *Helicobacter* isolates from 15 gastric mucosa samples of red foxes (*Vulpes vulpes*) shot by the hunters in the surroundings of Ljubljana, Slovenia. The isolation method that we developed and described previously was efficient even with 72-hour-old or frozen gastric mucosa samples. All seven isolates were confirmed as *Helicobacter* sp. by the genus-specific PCR. Whole-genome sequencing of all six isolates was carried out on the Ion Torrent PGM System. Based on the digital DNA–DNA hybridization and average nucleotide identity values, one isolate (L6) was identified as *H. felis*, whereas other six isolates belonged to three novel (yet to be characterized) gastric *Helicobacter* species. Core genome-based phylogeny showed that these isolates clustered with the sheathed gastric helicobacters. A comprehensive phenotypic characterization of the isolates including biochemical assays and MALDI-TOF MS is on-going. To our knowledge, this represents the first isolation of *Helicobacter* sp. from red fox and also the first isolation of gastric *Helicobacter* sp. from free-ranging carnivores in Europe.

Non-*pylori Helicobacters* (NHPHs) induce shifts on gastric microbiota in *Helicobacter pylori*-infected patients

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Introduction: Almost 12% of patients with gastric diseases co-infected with NHPH species and *H. pylori* (Liu *et al*.,2015). Among these NHPH species, four species (*H. suis*, *H. salomonis*, *H. felis* and *H. bizzozeronii*,) have been reported as the predominant organisms in human stomach.

Aim: To investigate the effect of NHPHs on the gastric microbiota.

Methods: In this study, we investigated the effect of NHPH species and *H. pylori* coinfection on stomach microbiota using 16S rRNA gene deep sequencing. Simultaneously, NHPH species-specific influence on the structure of gastric microbiota were indirectly characterized by comparing variation between *H. pylori* infection samples and co-infection samples.

Results and Conclusion: We found marked structural and functional variation of gastric microbiota between *H. pylori* infection samples and co-infection samples. Compared to *H.pylori* infection group, both *H.pylori* and *H.suis* co-infection (HPHS) group and *H.pylori* and H. *felis* co-infection(HPHF) group revealed the significant increase in phylotype richness and significant decrease in β diversity. The siginificantly decreased relative abundance of phylum *Firmicutes* and *Bacteroidetes* in HPHS group and HPHF group may be associated with the increase of predicted lipid metabolism pathways. To our knowledge, it is the first time to explore the effect of NHPHs species on the gastric microbiota.

Poster session
« Epidemiology and Public Health »

Use of genotyping tools to characterise and investigate the New Zealand Havelock North campylobacteriosis outbreak

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In August 2016 over 5,000 people became ill with campylobacteriosis following consumption of reticulated water in the small town of Havelock North, New Zealand. This paper describes the use of two genotyping methods (Multiplex Ligation-dependent Probe Amplification - Binary Typing [MBiT] and Whole Genome Sequencing [WGS]) to characterise *Campylobacter* isolated from the clinical cases, the water supply and suspected environmental sources. In the initial stages of the outbreak investigation MBiT analysis confirmed a linkage between cases and the water supply one day after receipt of the primary isolation plates, while WGS was completed three days later. Source attribution suggested that isolates were likely to be from ruminants. MBiT analysis was completed on over 200 isolates, allowing a rapid triage of isolates potentially linked or unlinked to the outbreak. WGS was then completed on 80 isolates with multi-locus sequence typing (MLST), whole genome MLST (wgMLST) and whole genome single nucleotide polymorphism (wgSNP) analysis used to compare isolates.

Three different genotypes of *Campylobacter* were isolated from the reticulated water supply (ST42, ST1517 and ST50), and were also found in 55% of clinical cases. Another 27% of clinical cases had ST3610 or ST474 genotypes. STs ST3610, ST3610 and ST42 were also found in faecal isolates from sheep in a nearby paddock. Within each ST, wgMLST identified less than 5 loci differences among isolates from clinical cases, water and sheep. This close similarity was confirmed using wgSNP analysis.

This large outbreak has showed the utility of MBiT analysis for rapid genotyping, including for isolates that were no longer able to be cultured. The WGS based techniques wgMLST and wgSNP were then able to confirm the close genetic relationships between isolates, and distinguish them from epidemiologically unrelated isolates. Both genotyping methods were highly amenable to practical usage in an outbreak situation.

Application of Whole Genome Sequencing for real time investigation of a *Campylobacter* outbreak associated with consumption of raw milk

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Campylobacter is the most common bacterial cause of gastroenteritis in England and Wales with over 600,000 incident cases per year. Nevertheless, common source outbreaks are rarely detected and most are associated with the consumption of undercooked poultry and unpasteurised dairy products.

In 2016 an outbreak of campylobacteriosis associated with the consumption of raw milk was detected in the UK. Isolates of *Campylobacter* spp. recovered from the faeces of 13 patients who were spatio-temporally linked to the outbreak and from the implicated raw milk were submitted to the Public Health England, Gastrointestinal Bacteria Reference Unit (GBRU) for species identification and molecular typing by Whole Genome Sequencing (WGS).

Genomic DNA was extracted from pure cultures using the Qiagen QIAsymphony SP automated nucleic acid purification system. WGS was performed using Illumina technology. FASTQ reads were quality trimmed and a K-mer identification step used to identify strains to species level. The 7-loci MLST profile and fine sequence typing by analysis of Single Nucleotide Polymorphims (SNPs) were determined using in-house bioinformatics software.

Results confirmed that 2 isolates from the raw milk and 7 human cases were *C. jejuni* MLST Sequence Type (ST) 7432. Furthermore, SNP analysis showed these strains to be identical. Six of the human cases had a history of raw milk consumption; the other case could not be contacted. An additional isolate of *C. jejuni* ST7432 that was closely related (1 SNP different) to the outbreak strain, submitted to GBRU two months earlier, was identified from a patient who was not part of the outbreak. Subsequent epidemiological investigations did not uncover any history of raw milk in this previous case.

This investigation marks the first real time use of Whole Genome Sequencing by the GBRU to microbiologically detect and confirm an outbreak of *C. jejuni* in the UK.

Consecutive waves of human *Campylobacter jejuni* infection associated with poultry identified by integrated whole genome surveillance in Luxembourg

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Campylobacteriosis has increased in Luxembourg during recent years with an incidence of 155 cases per 100 000 inhabitants in 2014, among the highest in the European Union. Due to *C. jejuni* accounting for 90% of cases, we conducted whole genome surveillance of *C. jejuni* over a three-year period 2014-2016 to determine which reoccurring genotypes infect humans, their distribution over time and their likely source.

Over a 3-year period, all isolates of *C. jejuni* (N=1 499) referred from clinical diagnostic laboratories in Luxembourg were initially genotyped by Sanger sequencing of *porA* and *gyrA* genes. All non-sporadic human isolates with porA-gyrA allele combinations occurring at least 3 times (N=695 representing 46.4% of all clinical isolates) and an additional set of 100 poultry and 41 ruminant isolates sampled during the same period were further processed by whole genome sequencing (WGS) on Illumina Miseq. A core genome MLST scheme of 637 genes (Ridom SeqSphere+, Germany) was applied to classify isolates into cluster types (CT) using a cluster distance of 13 alleles.

Human isolates were classified into 284 CTs. 21 predominant CTs occurring more than 5 times represented 48.6% of the sequenced isolates and 22.6% of all clinical isolates. The three most frequently occurring CTs containing 75, 31 and 28 clinical isolates, respectively, all displayed an epidemic patterns. Poultry isolates were represented in 14 and ruminant isolates in 3 of the 21 predominant CTs.

Whole genome surveillance of *C. jejuni* showed that approximately a quarter of human cases is caused by recurring genotypes displaying either endemic or epidemic patterns. Poultry rather than ruminants are most strongly associated with epidemic genotypes. New poultry-associated *C. jejuni* genotypes appear to emerge regularly in consecutive waves.

Whole genome sequencing and empirical epidemiology identifies disparate etiologies between *Campylobacter jejuni* sequence types ST50 and ST61

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Introduction: Traditionally infectious disease agents have been studied at the epidemiological level as one species or sub-species. However, this is changing with the developments in typing and whole genome sequencing (WGS) and here we describe the different etiologies of *C. jejuni* ST50 and ST61.

Materials and methods: WGS of campylobacter isolates from clinical cases (n= 1959), chickens (n=824), turkeys (n=117) and ruminants (n=559) from NE Scotland were obtained from Apr 2011 to Mar 2015. Pangenomic analysis was used to identify single nucleotide polymorphisms which enabled isolates to be compared and phylogenies to be constructed. Matching empirical epidemiological data (date, age, location, symptoms) were also obtained.

Results and Discussion: ST50 and ST61 comprised 6.8% and 5.7% of the animal isolates respectively. ST50 was over-represented in chicken and turkey but under-represented in ruminants. The opposite was the case for ST61. ST50 comprise 7.7% and ST61 1.6% of human cases. ST61 exhibited higher seasonality and higher incidence in the rural population than the other campylobacters. ST50 exhibited lower seasonality and relatively higher incidence in urban areas. ST61 was relatively more common in children aged 5-14 years. Diarrohea lasting >7 days was more common in ST61.
ST50 was only found once in sheep and once in cattle. If was found that at least one ST50 chicken isolate clustered within 0 SNP distance and 21 days with 13 human isolate. This corresponds to 8.6% of human ST50 isolates. Even though ST61 is rare in chickens but more prevalent in cattle and sheep, there is no evidence that chicken or other animal strains clustered with humans using the same genetic and temporal resolution as above.

Conclusion: ST50 is predominantly found in chickens and turkey whilst ST61 is usually found in ruminants. This leads to different routes of human exposure and correspondingly different epidemiological patterns of disease.

Molecular Characterisation of *Campylobacter* in Humans and Chicken from Nigeria

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Introduction: Livestock husbandry within close human habitation creates major public health risks for zoonoses. This practice is common among rural and urban dwellers in Nigeria. Domesticated animals including poultry and cattle as well as a range of wildlife, have been recognised as natural reservoirs for the zoonotic pathogen *Campylobacter*. A study was designed to culture *Campylobacter* from chicken and human sources and to epidemiologically characterise them.

Materials and Method: Sampling and culture was carried out from June 2013 -September 2016. DNA extraction was done on all the Isolates and then speciated by PCR. WGS was performed on an Illumina HiSeq 2000 sequencer with the 100 base paired-end sequences assembled into contigs using Velvet assembler. The genomes were uploaded to BIGSdb and 7 locus MLST determined.

Results: Dominant species were found to be *C. coli* and *C. jejuni*. Among the 171 isolates recovered, 47 ST's were identified from chicken sources and 33 from human sources, with 9 ST's common to both. Evolutionary history between these common ST's was reconstructed using whole genome SNP phylogeny along with molecular attribution of the sources of the human isolates.

Conclusion: The relationship between *Campylobacter* from chicken and human sources is discussed. This study is the first detailed examination of the sources of human *Campylobacter* cases in Nigeria.

Molecular characterization and antimicrobial resistance of *Campylobacter coli* strains isolated from different sources in Brazil

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Campylobacter coli is an important causative agent of human diarrheal diseases worldwide. In Brazil, *C. coli* is not frequently studied. Therefore, studies that molecularly characterize strains of this species and assess their antimicrobial resistance profiles are important. A total of 63 *C. coli* strains isolated from humans (12), animals (21), the environment (20) and food (10) between 1995-2011 in Brazil were studied for the presence of 16 virulence genes by PCR. The resistance profiles were obtained by MIC for erytromycin, ciprofloxacin, tetracycline and doxacycline. Furthermore, 40 selected *C. coli* strains were typed by MLST. All the strains presented the genes *fla*A, *cadF* and *sodB*. The *cdtB*, *flh*A, *dnaJ* and *pldA* genes were observed in 20, 11, 10 and 7 strains, respectively. The *cdtA* and *docA* genes were observed in two strains; the *cdtA* and *crsA* were detected in one strain and the *ciaB*, *wlaN*, *virB11*, and *racR* gene were not detected. The resistance profiles showed that 21 strains (33.3%) were resistant to at least one antimicrobial agent. The strains were typed by MLST in 28 STs of which six STs (ST 830, ST 1096, ST 1145, ST 1166, ST 1581 and ST 7370) were shared with strains isolated worldwide. The others 22 STs (ST 7628, ST 7713 - ST 7727, ST 8155 – ST8159 and ST 8161) comprised strains exclusively from Brazil. The presence of important virulence genes indicates the pathogenic potential of those strains. The existence of STs shared between the Brazilian strains studied and isolates of different countries is alarming since it may suggest that a possible contamination may have occurred. Furthermore, the antimicrobial resistance observed enhances this problem once a possible spread of resistance among bacteria is of public health concern.

Evaluating the epidemiological concordance of cgMLST clusters using the EpiQuant framework

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Although considerable progress has been made towards improving our understanding of the epidemiology of campylobacteriosis, key questions remain unanswered, hampering prevention and control efforts. These include the relative contribution of non-poultry reservoirs of *Campylobacter* towards overall burden of illness and the vast number of cases of campylobacteriosis that appear to be non-outbreak related.

In previous work, we developed a framework (EpiQuant) for systematically assessing the epidemiological concordance of subtyping clusters. In this work, we have used the EpiQuant framework to assess the underlying epidemiological characteristics of genotypic clusters derived from the analysis of Core Genome Multi Locus Sequence Typing (cgMLST) data on over 3,000 *C. jejuni* genomes from environmental, animal, and clinically-derived isolates.

Our results show that although increasing the similarity threshold for cgMLST cluster definition yields clusters with increasing epidemiologically and genetically concordant characteristics, this comes at the expense of an increasing proportion of strains that cannot be assigned to multi-isolate clusters, which can adversely affect source attribution. We have applied the EpiQuant framework for similarity threshold optimization and have used it to identify instances where genetic similarity estimates deviate from those expected based on underlying epidemiology. Moreover, we show how the genetic and epidemiological linkage between *C. jejuni* isolates can break down under specific circumstances, suggesting alternate transmission pathways for certain important *C. jejuni* genotypes.

Whole-Genome Sequencing (WGS) has the potential to revolutionize our study of the epidemiology of campylobacteriosis through its application in the routine analysis of *C. jejuni* isolates collected through human and non-human surveillance. We show that the EpiQuant framework can be used to optimize similarity thresholds for the interpretation of *C. jejuni* WGS data to facilitate attributing the likely source of human infections in the context of epidemiological investigations.

Genetic Diversity of *Campylobacter upsalienisis* Strains Isolated in the US between 1989 and 2016

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Background: *Campylobacter upsaliensis* has been isolated from blood and stool from humans and from feces of domestic pets. In previous MLST studies, there was substantial genetic diversity in *C. upsaliensis* from humans and dogs but insufficient data to establish whether dogs are a source for human disease. In this study, the genetic diversity of *C. upsaliensis* isolates from cases of human illness in the US received by CDC between 1989 and 2016 was examined by seven gene multi-locus sequence typing (MLST) and ribosomal MLST (rMLST).

Methods: *C. upsaliensis* isolates were sequenced using the Illumina MiSeq or HiSeq and assembled and analyzed using a BioNumerics 7.6 *Campylobacter* WGS database developed in collaboration with domestic and international partners. Genetic diversity was examined by comparing concatenated MLST genes from US isolates and isolates in the PubMLST *C. upsaliensis* database (http://pubmlst.org). All US strains were compared by rMLST; strains with identical MLST and rMLST types were further compared by whole genome analysis.

Results: MLST analysis of the US isolates showed novel alleles (n=190) and STs (n=130) for 139/145 isolates. Only 6 isolates had previously described STs. Substantial diversity was observed across all strains; human and dog isolates from the US, UK, and Finland intermingled throughout the tree. The US isolates frequently grouped differently by rMLST and MLST. However, pairs of isolates (n=5) with the same MLST STs consistently grouped together by rMLST; each pair were indistinguishable by whole genome analysis.

Conclusions: As in previous studies, there was considerable genetic diversity amongst human *C. upsaliensis* isolates from the US. The clustering by MLST of human and canine *C. upsaliensis* isolates suggests that dogs may be a zoonotic source of infection. rMLST shows promise as a useful tool for examining genetic diversity in *C. upsaliensis* and for investigating the epidemiology of *C. upsaliensis* infections in humans.

A study elucidating the socio-demographics of *Campylobacter* infection in Scotland

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Introduction: Previous work in Scotland has established that there is an apparent lower incidence of reported *Campylobacter* infections in deprived populations. This is not observed in hospitalised cases. This work investigates the origin of these differences between deprived and less deprived populations.

Materials and Methods: Retrospective *Campylobacter* cases (n=26,367) and hospital discharge (n=3,697) data (Jan 2012- Feb 2016) were analysed by descriptive and analytical epidemiological methods against a number of factors (deprivation, geographical location, age, gender, population density, properties on private water supplies (PWS) and farm animal numbers). The results from case data were compared with findings from a previous study (2000-2006).

Results: The incidence of campylobacteriosis was higher during Jan2012-Feb2016 (116±4 cases/100,000) than in the 2000-2006 study (97 ± 9 cases/100,000). There was an excess of cases (19%) in the least deprived compared with the most deprived populations (26% excess in the previous study). In both studies the incidence was higher during the summer, in those over 50 years of age, in males and in rural areas.
The incidence of *Campylobacter* hospital discharges was significantly higher in regions with high deprivation than in those where deprivation is low. Also, hospitalisation rates were higher during the summer and in the elderly, but lower in rural areas.
Multivariate logistic regression showed that there were regional differences in the number of cases by deprivation status, which were amplified in the older population. The least/most deprived case ratio was lower in children <5 years old compared with >65 years old. There were proportionately more least deprived cases in rural and peri-urban areas and PWS were a risk factor for least deprived, especially in regions with high sheep densities.

Conclusion: Differences in campylobacteriosis by deprivation exist. Whether this is real or is an artefact of reporting is the subject of further investigation.

Domestic campylobacteriosis in Scandinavia from 2000 to 2015: trends in time and space

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Introduction: Campylobacteriosis is the most frequently reported food- and waterborne infection throughout Europe, and notification rates in Denmark, Finland, Norway and Sweden have continuously exceeded the European average. It is unclear whether these high levels represent improvements in national surveillance systems and diagnostics, 'true' infection levels or a combination of both. Further, the role of geographical and demographic risk factors in determining disease patterns is poorly understood. Here, we describe the demographic and geographic trends in domestically acquired campylobacteriosis in four Scandinavian countries from 2000 to 2015.

Materials & Methods: Data on age, gender, geographical location (municipality level) and laboratory date for domestically acquired *Campylobacter* in the four countries from 1st January 2000 until 31st December 2015 (Norway until 31st December 2014) were used in combination with demographic and geographical background variables.

Results: Average incidences of campylobacteriosis ranged from 28.5 cases/100.000 population in Norway to 61.8 cases/100.000 population in Denmark. Males, children aged 0-4 years and persons aged 20-29 years had higher rates of infection in all countries apart from Finland. In all years, disease incidences increased from mid-May (all countries) and peaked in mid-July to early August; earliest in Finland (July) and latest in Denmark (August). Overall incidences were higher in densely populated municipalities, however children <10 years living in municipalities with low population densities were at higher risk of infection. In Norway and Sweden, southern and coastal municipalities had significantly higher rates of campylobacteriosis.

Conclusion: Campylobacteriosis remains the most prevalent bacterial gastrointestinal illness in Denmark, Finland, Norway and Sweden. We show that males, young adults and children living in rural areas are at higher risk of infection, highlighting these as suitable targets for infection prevention campaigns. Environmental risk factors, such as local climate and proximity to water, are also likely to impact the incidence of *Campylobacter* in Scandinavia.

What drives seasonality of human campylobacteriosis: dynamics of source contamination or of exposure to sources?

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Introduction: Human campylobacteriosis shows a clear seasonality in temperate countries. Several explanations for this seasonality have been provided but the exact mechanisms are still unravelled. The study aimed at testing the influence on campylobacteriosis seasonality of the seasonality of the contamination of and of the exposure to the main source of the two important routes of Campylobacter transmission: chicken meat (foodborne route) and natural water (waterborne route).

Methods: Time series analyses were applied to data collected through an integrated surveillance system in Canada in 2005-2010. Data included sporadic, domestically-acquired human cases of Campylobacter jejuni infection, contamination of retail chicken meat and of surface water by C. jejuni in the same area, and exposure to each source through barbequing and swimming in natural waters. The seasonality of each variable was modeled using Fourier series. Regression with autocorrelated errors was applied. The series were stationarized and stationarity was tested. Finally the link between the stationarized series was tested using transfer functions.

Results: Seasonal patterns were evident in all variables with a peak in summer for human cases and for both exposures, in fall for chicken meat contamination, and in late fall for surface water contamination. Time series analyses showed that the observed campylobacteriosis summer peak could only be significantly linked to risk exposures rather than sources: swimming rather than water contamination and barbequing rather than chicken meat contamination.

Conclusion: The results suggest that the summer increase in human cases may be more the result of amplification through more frequent risky exposures rather than the result of an increase of the Campylobacter source contamination.

Finding outbreaks in the ever-changing notification haystack: campylobacteriosis surveillance in two regions in New Zealand

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In August 2016, a large water-borne outbreak of campylobacteriosis occurred in Havelock North in the Hawke's Bay region of New Zealand resulting in an estimated 5,000 cases of disease from a population of 15,000. In such large outbreaks, identifying the common exposure is possible as a wealth of epidemiological information are available from the large number of cases. With knowledge of the exposure, future risk may be reduced or eliminated through targeted mitigation. However, most cases of food and water-borne campylobacteriosis in New Zealand and worldwide are due to isolated events where only a small number of people get sick. In such situations, identifying a common exposure to link cases can be difficult due to sparse epidemiological information. One technique is to model the underlying spatial and temporal trend in notification rates, with a spatio-temporal field of outbreak indicators over and above these trends. Such a model allows potential outbreaks to be detected whilst simultaneously modelling how temporal and spatial trends vary through time. We apply this model to the Manawatu and Hawke's Bay regions of New Zealand, showing how case rates in urban areas reduced significantly following intervention in the poultry industry, and that the temporal trend of notifications in these regions are similar despite their differing climates. In addition, we show that the majority of potential outbreaks in Hawke's Bay prior to the large outbreak in August 2016 also occurred in the town of Havelock North.

Human campylobacteriosis resulting from continuous source outbreaks in the United Kingdom

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Introduction: The majority of human campylobacteriosis cases are attributed to sporadic infection. Identified continuous source outbreaks have usually been associated with contaminated water where geographical clustering supports detection. More temporally and geographically diffuse outbreaks, due to the wide distribution networks of many foodstuffs, may be more common than previously thought. We demonstrate that such outbreaks can be detected using large scale human surveillance that includes pathogen genome analysis.

Materials and Methods: *Campylobacter jejuni* and *C. coli* human disease core-genome MLST (cgMLST) loci (1,343) were identified in 1,355 clinical isolates from two United Kingdom (UK) sentinel surveillance sites, using BIGSdb. Isolate clusters with five or fewer cgMLST allelic differences were identified and subsequently compared with cgMLST profiles from more than 8,000 whole genomes within the PubMLST.org/campylobacter database.

Results: Analysis of the 1,284 isolates identified that 692 (52%) isolates belonged to single-linkage clusters, of between two and 33 core genome allelic profiles, which differed at up to five, of a potential 1,343 cgMLST loci. Larger clusters, that were present across both surveillance sites, were found to have similar levels of genetic similarity to other genomes contained in the PubMLST.org/campylobacter database, notably those isolated from poultry, and from human disease dating back over an eight year period.

Conclusions: Our findings show that many apparently sporadic cases may be part of geographically continuous source outbreaks. These data also demonstrated evidence for the temporal stability of genotypes, and the identification of the same lineages causing human disease clusters in potential source reservoirs, particularly poultry.

Exploring *Campylobacter* surveillance data with visual analytics

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Introduction: Infectious disease surveillance data are multi-dimensional and complex. The increasing accessibility of whole-genome sequencing (WGS) offers new opportunities for pathogen surveillance, but conventional analysis approaches are inadequate for such complex data; therefore, new tools are needed. The field of visual analytics enables the exploration of complex datasets by combining analytical reasoning with interactive visual interfaces. This approach facilitates detection of expected trends and, more importantly, discovery of the unexpected. In this study, we show how visual analytics can be used to gain insights from the *Campylobacter jejuni/coli* PubMLST database (http://pubmlst.org/campylobacter/).

Methods: We developed an interactive visual analytics dashboard, synthesising provenance, phenotype, and genotype data from the PubMLST database. One of the strengths of using a visual analytics approach is the ability to integrate, explore, and analyse data from multiple sources. To demonstrate this, host source attribution data for a subset of clinical isolates were also integrated into the dashboard.

Results: The interactive dashboard provided an intuitive and informative overview of the 50,793 isolates present in the database. It was used to filter and zoom in to investigate subsets of isolates of interest. As a case study, surveillance data for 8,726 *Campylobacter* isolates collected between 2003 and 2017 in Oxfordshire, UK, were explored. The dashboard summarised multilocus sequence typing data alongside provenance data, also incorporating WGS data, which were available for 57% of the isolates. Trends, changes, and anomalies in descriptive and molecular epidemiology were easily identified using this approach, including for source attribution.

Conclusions: Surveillance data must be rapidly and easily analysed and understood to develop public health interventions, allocate resources, and inform policy makers. We demonstrated how visual analytics can be used to support these activities. The approach developed for this study is broadly applicable and could readily be extended to other pathogens.

Chicken persists as the dominant source of *Campylobacter* in the United Kingdom (UK)

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Introduction: Genetic attribution models are widely used to estimate the contribution of different host sources to human campylobacteriosis. However, the impact of inaccuracy has not been robustly assessed. We measured accuracy using self-attribution and used these validated reference datasets to quantitatively estimate sources of *Campylobacter jejuni* and *Campylobacter coli* infections longitudinally and in two populations in the UK.

Methods: Animal and environmental *C. jejuni* and *C. coli* isolates with multilocus sequence typing (MLST) data were mined from the published literature. The resulting reference sets were validated, measuring accuracy of attribution for isolates from known sources. Sequenced human disease isolates collected at two UK sentinel sites, Oxfordshire (2003-2016) and Newcastle/North Tyneside (2015-2016), were assigned to host sources using the STRUCTURE algorithm.

Results: Reference sets of 8,968 *C. jejuni* and 3,967 *C. coli* isolates were established, comprising ten and seven sources, respectively. Simplified reference sets, including only major animal sources, were most accurate (73-91% accuracy for *C. jejuni*; 65-78% for *C. coli*). MLST-based attribution of 7,581 *C. jejuni* and 801 *C. coli* disease isolates from Oxfordshire identified chicken as the primary source of infections between 2003 and 2016 (55% *C. jejuni*; 46% *C. coli*). Ruminants accounted for most of the remaining isolates for both species. There was little variation in host sources over time. Chicken and ruminants were also the predominant sources among 1,282 *C. jejuni* and 121 *C. coli* isolates collected in Oxfordshire and Tyneside between 2015 and 2016, although there were some differences in sources for these two populations.

Conclusions: Chicken and ruminants represent persistent and widespread sources of *Campylobacter* in the UK, although with some regional differences. Longitudinal attribution of clinical *Campylobacter* can monitor the impact of interventions even when surveillance coverage is inconsistent. Resources developed for this study are publicly available to improve implementation of routine source attribution.

Chickens in the city, cows and sheep in the country:
A simple model for the source of campylobacteriosis

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An important public health question is determining the source of food or environmentally acquired diseases such as campylobacteriosis. In the absence of epidemiological information for each human case, the use of genomic information, such as multilocus sequence type, allows attribution of cases to sources by comparing the types observed in human cases to the types observed in food and environmental samples. Several statistical models are available for attribution, all of which involve estimating the distribution of sequence types on each source. The asymmetric island model models the sequence type distributions by estimating evolutionary parameters (mutation and recombination) in addition to migration rates between each source. A much simpler model is to use a Dirichlet distribution, informed by prior information and the observed types on each source, to model the sequence type distributions. We show that this simple model provides similar information as the island model for sequence types that are observed frequently, and that the island model provides additional information only for types that have not been observed frequently (or at all) among sources. Where epidemiological information is also available for cases, we show that attribution with either model may be improved. Using surveillance data from the Manawatu region of New Zealand, we show that the relationship between rurality and poultry or ruminant attribution may be modelled by a linear trend: cases from very rural areas are likely ruminant associated, cases from highly urban areas are likely poultry associated.

Calves as source of infection with *Campylobacter* in a farm worker

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Introduction: The number of campylobacteriosis cases has increased in Sweden as well as in many other European countries. Poultry and poultry meat are the most common sources of campylobacteriosis but other domestic animals are of significance. Outbreaks have been reported among farm workers and visitors. A farm worker, caring for the youngest calves of a farm in southern Sweden got severely ill with campylobacteriosis and was hospitalized.

Material and methods: Stool sample from the farm worker were analysed using standard microbiological methods including culture on *Campylobacter* selective agar. In an ongoing project, different types of samples were assessed in order to identify an optimal sample type for sampling dairy farms. From the farm, milk filters (n=7) and cattle faeces (boot sock) (n=20) and faecal pat (n=31) samples were collected at six sampling occasions from three different age groups; dairy cows (n=21), heifers (n=14) and calves (n=23) ≤12 months. Faecal samples were analysed by direct plating on mCCDA and the sock and milk filter samples were analysed by enrichment in Bolton broth and culture on mCCDA according to ISO 10272. Isolates were further typed using PFGE.

Results: The human isolate was identified as *C. jejuni.* Thirty-one of the 58 farm samples were positive for *Campylobacter*: one was identified as *C. coli,* three as *Campylobacter* sp, six as *C. hyointestinalis* and 21 as *C. jejuni*. Two calf isolates of *C. jejuni* and the isolate from the farm employee had identical PFGE profiles.

Conclusions: Cattle are often colonized with *Campylobacter* spp., *C. jejuni* is the species most commonly isolated from human cases as well as from cattle. Persons who work with cattle may be at increased risk of exposure to *Campylobacter*. This should be considered during the ordinary work at dairy farms and visits at farms especially by children.

Insight into Consumer Phase Practices Regarding Chicken Meat Consumption, Handling, and Preparation in Egypt

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In Egypt, the overall incidence of *Campylobacter* diarrhoea, especially in rural areas, is considered among the highest incidence rates ever reported from a developing country. The gap of knowledge about incidence of *Campylobacter* in chicken meat, combined with the scarcity of data on consumer practices regarding chicken meat handling, preparation and consumption hinders accurate assessment of the human health burden of this important zoonotic pathogen in Egypt.
A food safety questionnaire was administered through face-to-face interviews with 200 consumers in Alexandria, northern Egypt. The questionnaire designed to capture information on chicken meat consumption, handling, and preparation practices that could be useful to update the development of quantitative risk assessment model. The most frequent consumption pattern of chicken meat (in 45% of the interviewee) was 2 times/week. In addition, the typical serving size among adults was a quarter of chicken among 84.5% of the interviewed consumers. The majority (78%) of consumers indicated supplying their chickens from the poultry shops at wet markets, while fewer (6%) consumers prefer buying frozen chicken from supermarkets. Interestingly, 73% of the consumers view frozen chickens as inferior in quality to freshly slaughtered chickens sold at the wet markets.
Regarding consumers handling and preparation practices; the entire interviewed consumers indicated that they wash chickens in kitchen's sink prior to cooking. Washing chicken with running hot water in a pot was the preferred method among 40% of the interviewee. Washing hands after handling chicken was "a very important to consider practice" among 90% of consumers. Added to that, 63% of the consumers indicated considering changing cutting board after preparing chickens as "a very important to consider practice". This study provides insightful information about variability in chicken meat consumption pattern and serving size among consumers. This work also highlighted some risky cross-contamination related practices. The output from this study could be used to develop a reliable quantitative risk assessment of the foodborne pathogen *Campylobacter* in Egypt.

Modelling *Campylobacter* infection dynamics in young children in low-resource settings

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(A)symptomatic infections with thermophilic *Campylobacter* spp. are highly prevalent among children in developing countries and have recently been implicated as one of the key factors responsible for environmental enteric dysfunction and stunting in young children. Previous efforts of modelling Campylobacter infection dynamics describe the effect of boosting and waning of immunity in exposed populations, and have suggested that force of infection and probability of asymptomatic infection depend on the exposure frequency and dose. In these studies, parameter estimates were based on the only available experimental challenge-rechallenge study. Due to scarcity of experimental data and observational studies in low-income countries, applying these compartmental models to understand Campylobacter dynamics is still in its infancy. The current study uses recently published observational data on Campylobacter prevalence in diarrheic and non-diarrheic children in the MAL-ED study to estimate model parameters in developing countries. Available data have been transformed to represent the different compartments of the model and were plotted for all eight reported countries for comparative analysis of disease dynamics. Estimation of model parameters was done with maximum likelihood methods. The current model can be used to predict the impact of changing the exposure frequency and dose on the prevalence of Campylobacter in children, to support hygiene interventions in low resource settings. It provides novel opportunities to estimate the incidence of pathogen-specific diarrheal illness in high exposure populations.

Colon cancer risk in patients with a history of *Campylobacter* infection

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Background: Colon cancer is a major cause of cancer morbidity and mortality worldwide. While an estimated 20% of the global cancer burden is attributable to infectious agents, only a few bacterial infections are associated with cancer development, such as *Helicobacter pylori* with gastric cancer and MALT lymphoma, and *Salmonella* Typhi with gallbladder carcinoma. Recent research also showed that *Salmonella* Typhimurium infection facilitates colon cancer development in genetically predisposed mice. We examined whether *Campylobacter* infection, the primary cause of bacterial gastroenteritis in industrialized countries, is associated with increased colon cancer risk in humans.

Methods: National infectious disease surveillance records (2002-2015) for Dutch residents aged ≥20 years at *Campylobacter* infection (n=25,316) were linked to colon cancer records in the Netherlands Cancer Registry. *Campylobacter* infection is generally laboratory-confirmed under medical consultation after 1-2 weeks of illness. Colon cancer risk after campylobacteriosis was compared with expected risk based on cancer incidence in the general population. Effects of age at infection, gender, follow-up time, and infecting *Campylobacter* species (*C. jejuni*, *C. coli*, or other) were also assessed.

Results: In total, 83 patients were diagnosed with colon cancer ≥1 year after diagnosis of *Campylobacter* infection. Compared to the general population, overall colon cancer risk among patients with a history of *Campylobacter* infection was not significantly increased (standardized incidence ratio (SIR) 1.06; 95%CI 0.84-1.31). A significantly increased SIR for colon cancer of 2.27 (95%CI 1.17-3.96) was observed only among those acquiring campylobacteriosis at 40-49 years of age. No significant effects of gender, follow-up, and *Campylobacter* species were observed.

Conclusions: Patients diagnosed with campylobacteriosis at least between 40 and 49 years of age have an increased risk of developing colon cancer later in life. The biological basis of this association still needs to be elucidated as to claim any contribution of campylobacteriosis to colon cancer development.

Increased risk of inflammatory bowel disease after *Campylobacter concisus* and *Campylobacter jejuni* infection; a Danish population-based cohort study

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Introduction: *Campylobacter concisus* and *Campylobacter jejuni* infection have been linked to increased risk of inflammatory bowel disease (IBD) after enteric disease. Here, we compared the risk of IBD in *C. concisus* and *C. jejuni* positive patients and age- and gender-matched population controls from North Denmark Region, Denmark.

Material and methods: Using data from health-care registries, we identified 3,208 patients with a first-time positive stool culture with *C. concisus* or *C. jejuni* during 2009-2013. We excluded 118 Campylobacter patients who had other pathogens isolated in fecal cultures, and their 1,180 matched comparisons. We also excluded 125 exposed individuals with IBD before or at index date and their 1,250 matched comparisons, plus another 237 comparisons with pre-existing IBD. Thus, 983 patients with *C. concisus*, 1,982 patients with *C. jejuni*, and 29,413 matched comparisons comprised the final study cohort. We used multivariable Cox regression to compute hazard ratios of hospital-diagnosed IBD among Campylobacter patients versus controls. The median follow-up was 45 months.

Results: On follow-up, a first-time diagnosis of IBD was reported in 30/983 (3.1%) *C. concisus* positive patient vs. 17/9,745 (0.2%) for matched comparisons. Among *C. jejuni* positive patients, 16/1,982 (0.8%) vs. 51/19,668 (0.3%) for matched comparisons, had a first-time diagnosis of IBD. The adjusted hazard ratio (95% CI) for IBD for *C. concisus* vs. comparisons was 20.3 (10.6-38.9) and for *C. jejuni* vs. comparisons it was 3.0 (1.7-5.4) for the whole period. If the first year after Campylobacter infection was excluded, the hazard ratios were 6.4 (2.3-18.0) and 1.6 (0.6-3.9) for *C. concisus* and *C. jejuni*, respectively.

Conclusions: Although we cannot rule out that detection bias may influence the estimates of risk, we demonstrated an increased risk of IBD in patients with both *C. concisus* and *C. jejuni* enteritis, with the highest risk among the *C. concisus* positive patients.

Campylobacter Pancolitis Mimicking Ulcerative Colitis

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Aims: Campylobacter colitis is the community-acquired acute bacterial infection and usually accompanied by fever, abdominal pain, and diarrhea. The majority of patients with Campylobacter colitis have some component of segmental colitis, usually beginning in the small bowel and progressing distally to the cecum and colon. Camyplobacter colitis is a rare cause of pancolitis. Here, we present a case of Campylobacter pancolitis in a young male with abdominal pain, diarrhea, and bloody stool mimiking ulcerative colitis.

Case Report: A 25-year-old male was admitted to the emergency room with a 5-day history of abdominal pain, diarrhea, and bloody stool. He had no fever and usually had normal defecation once in a day without diarrhea. He had no past medical history such as hypertension, diabetes mellitus, pulmonary tuberculosis, or hepatitis. On physical examination, abdomen was slightly protuberant and soft with active bowel sounds. Laboratory findings showed no specific results except increased ESR (16 mm/hr) and CRP (5.12 mg/dL). The initial chest and abdominal x-ray showed normal findings. However, on abdominal-pelvic CT, diffuse wall thickening for entire colon was observed (Fig 1). The colonoscopic finding revealed a loss of vascularity, edema, hyperemia, fine granules, and exudate diffusely from rectum to cecum (Fig 2). Especially, on the ileocecal valve, large shallow ulcer was noted (Fig 3). Two days later after colonoscopy, the stool specimen revealed positive *Campylobacter* PCR result. He was improved with conservative management including antibiotics and dischared 5 days ater hospitalized.

Conclusions: Campylobacter colitis is recently increased and rarely could mimicking ulcerative colitis in cases of pancolitis with bloody stool. However, we could distinguish Campylobacter pancolitis from ulcerative colitis, especially ulceration on the ileocecal valve was observed.

CHRO are highly relevant with the Global Water Pathogen Project

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The Global Water Pathogens Project (GWPP) updates knowledge on enteric pathogens (including among others *Campylobacter*, *Helicobacter* and *Arcobacter*) using advanced information technologies disseminates a state-of-the-art reference work, replacing Sanitation and Disease Health Aspects of Excreta and Wastewater Management (by Feachem, Bradley, Garelick and Mara. 1983), and creates an online open-access publication data base (www.waterpathogens.org).
The audience of GWPP are policy makers, community leaders, public health officials, and in engineers (i.e., those making decisions on the efficiency of sanitation technology to control pathogens and protect public health). One of the goals for each chapter is to make the information really accessible for the user community and to provide quantitative data needed for risk assessment. Considering that the genera mentioned above are all the targeted interest of the CHRO 2017 19th International Workshop we considered relevant to disseminate this initiative platform among participants.
The epidemiology, transmission, persistence, survival, and wastewater treatment reductions of *Campylobacter*, *Helicobacter*, and *Arcobacter* were reviewed and each individual chapter focused on mapping potential pathways of the pathogens to humans through human, animal, food, and environmental reservoirs with a focus primarily on sewage/feces.
Each chapter summarizes the above findings and provide a general description of *Campylobacter*, *Helicobacter* and *Arcobacter* as well as tables showing persistence and survival in various water matrices, as well as concentration reductions in various wastewater treatment processes. The data summarized in this project can be used in qualitative and quantitative microbial risk assessments or to simply better inform treatment and policy decisions.
To bring awareness to the GWPP project and goals, promote dissemination of information, create consistency of assumptions used in microbial risk assessments (such as concentrations, treatment reductions, etc.), identify data gaps for each bacteria, and develop a roadmap for future research to fill data gaps.

Poster session
« Methods for Detection, Identification and Characterisation »

Development of "Recommendations for the Diagnosis of Campylobacter Infection by Clinical and Public Health Laboratories"

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Background: Diagnosis of *Campylobacter*-associated acute diarrheal illness is complicated by its fastidious growth requirements. Increasingly, laboratories are using culture-independent diagnostic tests (CIDT) for *Campylobacter* detection. To address these issues, the Centers for Disease Control and Prevention (CDC), the Association of Public Health Laboratories (APHL), and American Society for Microbiology (ASM) convened a workgroup to develop recommended guidelines for the diagnosis of *Campylobacter* infection. The goal of these guidelines was to improve knowledge and practices for *Campylobacter* diagnosis and strengthen the partnerships between clinical and public health professionals to reduce the burden of *Campylobacter* disease.

Methods: Discussions for developing guidelines began in June 2012. Subgroups with experts from public health, clinical laboratories, and regulatory agencies drafted specific sections of these recommendations. In 2014 the sections were merged and sent out for initial comment and revision. In November 2016, the chairs and co-chairs from each subgroup re-convened to review and update the drafted document. Final consensus for recommended best practices and a strategy for finalizing these recommendations was established at this meeting.

Results: The "Recommendation for the Diagnosis of *Campylobacter* Infection by Clinical and Public laboratories" summarizes clinical features and public health perspectives for *Campylobacter* infection and recommends practices for specimen collection, transport, and testing using both culture and CIDT methods. Although diagnostic testing algorithms for *Campylobacter* infection will continue to evolve, this document provides a base of knowledge and information that can improve partnerships between clinical and public health professionals, thus improving *Campylobacter* diagnosis, treatment, and disease surveillance.

Conclusion: The "Recommendation for the Diagnosis of *Campylobacter* Infection by Clinical and Public Laboratories" provides guidelines to address the needs of clinical and public health laboratories for *Campylobacter* diagnosis. This document provides information to testing laboratories, regulatory agencies and key decision makers in the medical community regarding *Campylobacter* infection.

Using Pan-genomes for Molecular Assay Design:
Development of a Multiplex Ligation-dependent Probe Amplification (MLPA) Assay for the Detection of 28 Epsilonproteobacterial Taxa

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The Epsilonproteobacteria class includes three genera containing taxa known or suspected of causing human gastroenteritis. Although it is possible to detect these taxa using molecular techniques such as PCR, there is currently no single assay for the detection of taxa across the three genera. Large scale-BLAST score ratio analysis was used to evaluate the pan-genomes of *Arcobacter*, *Campylobacter* and *Helicobacter*. Candidate genes for MLPA design were identified for 28 taxa: 23 taxa on the basis of intra- and inter-taxon homology, length and putative function; one taxon using Delta-bitscore, a profile-based homology scoring method; and four taxa using published PCR data. MLPA probes were designed and manufactured for these 28 taxa and tested against a collection of 126 DNA extracts representing 59 Epsilonproteobacterial species and 15 human enteric bacterial pathogens. The MLPA probes were detected using capillary electrophoresis and analysed in BioNumercis using the AFLP module. Band matching assigned peaks to the probes on the basis of length and the results were exported as common-separated values. Concordant results were obtained for the majority of probes and DNA extracts. However, the *C. lari* subsp. *concheus* and *H. pullorum* probes failed to detect the target DNA; all of the urease positive thermophilic *Campylobacter* DNA extracts were also positive for the *C. subantarcticus* probe; and some probes lacked repeatability when the DNA was diluted in TE buffer containing 1 mM EDTA. In addition, optimisation of position tolerance settings for band matching within BioNumerics was required to ensure the majority of "spill-over peaks" – false-positive peaks caused by some of the true positive products being read as the next smaller product – were not assigned. Although not all of the probes provided sensitive, specific and repeatable results, and further optimisation is required, the MLPA assay shows promise for the simultaneous detection of a range of Epsilonproteobacterial taxa.

Characterization of the heme uptake regulator, HeuR, in *Campylobacter jejuni*

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Campylobacter jejuni is a leading cause of severe gastroenteritis due to its ability to reside in animal reservoirs and persist in the environment prior to infecting humans. Several post-infectious disorders such as Guillain-Barré syndrome and reactive arthritis also occur following infection with *C. jejuni.* As such, understanding the factors that influence Campylobacter's ability to colonize and survive in animal hosts is important to human health. We are interested in characterizing the heme uptake regulator, HeuR, of *C. jejuni* to determine its role in the colonization of animal hosts. We have found that HeuR, which contains a Per-Arnt-Sim (PAS) domain and a helix-turn-helix (HTH) DNA-binding domain, binds to the promoter region of the *C. jejuni* heme utilization (chu) gene cluster; the presence of HeuR is required for efficient utilization of heme as an iron source. Following purification of several HeuR truncations and employing isothermal titration calorimetry (ITC), we have observed that the PAS domain interferes with HeuR binding to the chu promoter region. This result suggests that, in its purified form, full-length HeuR is in a low affinity conformation, possibly due to a lack of ligand co-purification. Since it is currently unknown which ligand binds to the PAS domain of HeuR, we have utilized both mass spectrometry and x-ray crystallography to identify molecules that co-purify with HeuR. Further, using size-exclusion chromatography, we have found that HeuR exists primarily as a dimer in solution and we are determining whether this oligomeric state is required for DNA binding activity. Lastly, we have purified the heme receptor (ChuA) of the *Campylobacter* heme uptake system and are analyzing binding affinities of this protein for different porphyrins, including heme. This will further our characterization of HeuR, since it will offer insights into the homeostasis of various metals that are controlled by HeuR.

**Comparison of PFGE, MLST and MALDI-TOF,
for typing *Campylobacter coli*
and relation with the virulence of these strains**

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PFGE, MLST and MALDI-TOF were tested on 61 *Campylobacter coli* strains isolated from pigs in order to evaluate their discriminatory power and their agreement in the distribution of the strains. Moreover, we looked if the capacity of adhesion and invasion of the strains on *Caco2*-cells could be related to PFGE-, Sequence- or Maldi- types.

The PFGE-, Sequence- and Maldi- types obtained from the 61 strains were distributed in dendrogram under BioNumerics. To compare the diversity and the distribution of the strains in each method relative to each other, we previously clustered the K*pn1*-PFGE types in dendrogram with 70, 75 or 80% of similarity, and the Maldi-Types in dendrogram with 95, 97 or 99% of similarity. Using the "comparing partitions" method (http://www.comparingpartitions.info), we could – evaluate the discriminatory power of each method (Simpson's index of diversity) - measure the agreement between two-by-two methods (adjusted rank index and jackknife pseudo-values) and evaluate the predictiveness of a typing method toward the others (Adjusted Wallace coefficient). The strains were also classified according their level of pathogenicity using hierarchical clustering with the method "hclust ward D2" implemented in R.

The highest diversity was obtained for PFGE80% (ID=0.970) followed by Maldi99, PFGE75, MLST, PFGE70, Maldi97 and Maldi95 (ID=0.613). The highest agreement between two methods was between MLST and PFGE70 (p=0.336). Furthermore, two strains with the same Sequence type had 58% chance of belonging from the same Maldi95 cluster (14 on the 16 ST854 strains were in the MT5 cluster) and, 55% chance of belonging from the same PFGE70 cluster. Two strains that were clustered together by PFGE80 had 43.9% chance of belonging from the same ST and 42.6% chance of belonging from the same Maldi95 cluster. There was no agreement between the level of pathogenicity of the strains and the PFGE-, Sequence- or Maldi- types.

Evaluation of a core genome multilocus sequence typing scheme for *Campylobacter* outbreak investigation

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Introduction: *Campylobacter* is the main cause of acute bacterial gastroenteritis in humans in the European Union. Most cases of campylobacteriosis are sporadic cases, outbreaks seem to be rare. Subtyping of strains by whole genome sequencing is a new and promising tool for epidemiological surveillance and outbreak investigation. The aim of this study was to evaluate a core genome multilocus sequence typing (cgMLST) scheme for epidemiological typing of *Campylobacter.*

Material and methods: A total of 143 *Campylobacter* clinical isolates (121 *C. jejuni*, 22 *C. coli*) from sporadic cases collected in Austria in 2014 and 20 additional isolates (18 *C. jejuni*, 2 *C. coli*) from five outbreaks occurring between 2008 and 2014 were included in this study. Isolates were subjected to whole-genome sequencing using Illumina MiSeq sequencing technology and characterized by cgMLST using SeqSphere+ (Ridom, Münster, Germany). In addition, all outbreak isolates were analyzed by pulsed-field gel electrophoresis (PFGE) using restriction enzymes *Kpn*I and *Sma*I.

Results: Using a cgMLST scheme comprising 637 core genome targets and a cluster threshold of 13 allelic differences for cluster type (CT) definition all outbreak strains could be correctly assigned to the respective outbreak showing one or none allelic difference. This was in line with PFGE results showing identical restriction patterns. Of the 143 epidemiologically unrelated isolates 93 (65.0%) isolates showed a unique cluster type, while 50 (35.0%) *C. jejuni* isolates could be assigned to a cluster type comprising two or up to four isolates.

Discussion: In this study cgMLST proved to be an effective method for demonstrating a clear relationship between outbreak-associated *Campylobacter* isolates. However, as a high portion of isolates not known to be epidemiologically linked were assigned to the same cluster type a combination of WGS data and epidemiological data is crucial for epidemiological typing with regard to outbreak investigation.

Whole Genome Sequencing of Campylobacter for Routine Surveillance

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Food safety remains a public health priority as foodborne illness is a significant health service and economic burden. The Gastrointestinal Bacteria Reference Unit (GBRU) at Public Health England routinely detects and characterizes foodborne pathogens including *Campylobacter*, *Salmonella, pathogenic E. coli* and *Listeria* that are involved in outbreaks of gastroenteritis linked to the consumption of contaminated food products. With the advent of Whole Genome Sequencing (WGS), new methods have been developed to characterise these pathogens and provide the opportunity to replace many of the conventional microbiological techniques used to identify and type bacterial pathogens.

GBRU are currently using WGS for the routine identification and epidemiological investigation of *Campylobacter*. As with traditional typing a hierarchical approach is required to provide appropriate levels of discrimination depending on the clinical or epidemiological question at hand. This includes species, clone and strain identification and antimicrobial resistance characterization. We have used multiple bioinformatics approaches based on genome sequence analysis to provide characterization of foodborne pathogens at these differing resolutions implemented in an automated pipeline. Here we present data to demonstrate the applicability of different genome analysis methods including K-mer screens, traditional and core genome Multi Locus Sequence Typing, antimicrobial resistance characterization and Single Nucleotide Polymorphism analysis for the routine characterization of *Campylobacter* isolates.

The results illustrates how the implementation of WGS analysis is a viable alternative to conventional reference microbiological methods and demonstrates how WGS can enhance the detection, characterisation and investigation of *Campylobacter* outbreaks.

Evaluation of Whole Genome Sequencing for *Campylobacter jejuni* Surveillance and Outbreak Detection

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Background: *Campylobacter jejuni* is a leading cause of bacterial foodborne illnesses in the United States. Pulsed-field gel electrophoresis (PFGE) is currently used within PulseNet USA for *Campylobacter* surveillance and outbreak investigations. However, PulseNet USA is moving towards whole genome sequencing (WGS) for surveillance of all the pathogens it tracks, including *Campylobacter*. PulseNet USA is evaluating whole genome multi-locus sequence typing (wgMLST) for WGS-based cluster detection. In this study, we examined the ability of wgMLST to cluster or differentiate outbreak-associated and sporadic *C. jejuni* isolates indistinguishable by PFGE.

Methods: *Sma*I and *Kpn*I PFGE patterns for 140 *C. jejuni* isolates (83 isolates from nine epidemiologically-linked outbreaks and 57 sporadic isolates) were generated using the PulseNet *Campylobacter* protocol, analyzed in BioNumerics 6.6.10, and named using PulseNet naming guidelines. These isolates were sequenced using the Illumina MiSeq or HiSeq and the sequences were analyzed using the *Campylobacter* wgMLST v.4 allele database. The wgMLST database was developed in collaboration with domestic and international partners for *Campylobacter* surveillance. These results were compared with the high quality single nucleotide polymorphism (hqSNP) analysis results generated using the LYVE-SET pipeline (github.com/lskatz/lyve-SET).

Results and Conclusions: On average, 1561 (1347-1800) wgMLST loci were identified per genome among the *C. jejuni* sequences in this analysis. Outbreak-associated *C. jejuni* isolates indistinguishable by *Sma*I/*Kpn*I PFGE were differentiated from epidemiologically unrelated isolates by wgMLST analysis. These results were concordant with hqSNP analysis of the sequences. Our study shows that both wgMLST and hqSNP analysis provide greater resolution and epidemiological concordance compared to PFGE for *Campylobacter* surveillance and outbreak detection. Additionally, hqSNP analysis is dependent on a priori knowledge of isolates to select the correct reference genome before an analysis is run. However, selection of a reference genome is not required for wgMLST analysis making this method more user-friendly and requiring less specialized knowledge to perform.

Development and Standardization of a Whole Genome Multi-locus Sequence Type (wgMLST) Database for *Campylobacter* Characterization

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Background: The study of genetic diversity in *Campylobacter* is often limited to *C. jejuni* and *C. coli* strains due to their significance as human pathogens. The implementation of whole genome sequencing (WGS) at CDC has begun to replace the traditional phenotypic testing, PCR, phylogenetic analysis of single genes and PFGE with a unified WGS workflow for *Campylobacter* identification and subtyping. This allows for increased characterization of six *Campylobacter* species commonly associated with human disease (*C. jejuni*, *C. coli*, *C. upsaliensis*, *C. lari*, *C. fetus*, and *C. hyointestinalis*) received in the Enteric Diseases Laboratory Branch, CDC. In this work, we present the development and ongoing validation of a whole genome multi-locus sequence typing (wgMLST) system for the characterization and subtyping of these six *Campylobacter* species.

Methods: Closed or high quality draft genomes of a genetically diverse collection of *Campylobacter* species were provided to Applied Maths (bioMérieux) and were used to develop a wgMLST scheme containing over 24,000 loci. Using the BioNumerics v7.5 database platform, the robustness of the allele definitions was tested against 288 genome assemblies comprised of six *Campylobacter* species by incrementally decreasing homology thresholds until changes in phylogenetic topology and allele assignments were observed.

Results and Conclusions: Allele assignments for loci of all species tested increased as homology settings decreased with few loci reassigned different allele numbers and a 75% homology threshold determined to give the optimum utility. Among the six species examined, most loci with allele assignments were from strains of *C. jejuni* and *C. coli*. Additionally, there were consistent phylogenetic relationships between epidemiologically related *C. jejuni* and *C. coli* strains at all homology settings examined. This wgMLST scheme proved effective for characterization of *C. jejuni* and *C. coli*; however, future work will be needed to improve allele detection for the other species tested.

Something old, something new: added value of a vintage culture technique for emerging enteric pathogen detection.

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Introduction: Bacterial stool culture for *Campylobacter*, focused on the recovery of *Campylobacter jejuni/coli*, is gradually replaced by antigen and molecular based detection techniques. These tests offer rapid results with a higher yield, but do not allow susceptibility testing and are not agnostic. The aim of the study was to validate a feasible culture method with comparable efficiency to standard culture to complement these tests and above this allow the detection of other emerging *Campylobacter* and *Arcobacter* spp.

Materials and methods: The study included 800 consecutive fecal samples from patients with clinical suspicion of enteric infection sent to the lab of a Belgian secondary care hospital during winter 2016-17. Next to standard culture on selective Butzler agar with incubation at 42° C for 48 hours in microaerobic conditions, passive filtration of the stool suspension was performed using a 0.65 µm pore size membrane on horse blood agar with incubation for up to 7 days at 35°C in microaerobic conditions. A pilot experiment to retrieve *Arcobacter* spp. by filtration of overnight incubated selective Campylobacter broth was organized.

Results: Thirty *C. jejuni* and seven *C. coli* were recovered from both culture techniques. Seven *C. jejuni*, one *C. hyointestinalis* and one *C .coli* only grew on standard culture. Filtration technique yielded one additional *C. jejuni*, 21 *C. concisus*, three *C. upsaliensis*, two *A. butzleri*, one *C. curvus* and one *C. lari*. *A. cryaerophilus* and *A. butzleri* were easily retrieved after filtration of overnight incubated selective Campylobacter broth.

Conclusion: Sensitivity of the filter technique compared to standard culture for *C. jejuni/coli* is satisfactory and can be optimized further by the use of selective broths. Additional detection of emerging *Campylobacterales* in enteric and inflammatory bowel disease with the filter technique is evident. Combining rapid non culture techniques with filtration culture proves to have added value without significantly increasing the workload.

Orion GenRead Campylobacter.
A new kit for a rapid detection of Campylobacters in stools.

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Introduction: Campylobacter infections constitute the first cause of bacterial enteric infections. While culture is the usual diagnostic test used, it appears to lack sensitivity and requires a long delay before obtaining a result.
The goal of this study was to test prospectively on stool specimens the sensitivity, specificity and convenience of a new test based on the isothermal Strand Invasion Based Amplification-method (SIBA); Orion GenRead Campylobacter (Orion Diagnostica, Finland) targeting 16S rDNA in comparison to a composite reference.

Material & Methods: Stools obtained from patients seeking medical attention for enteric infections at the Emergency Ward of our hospital (children and adults) during the summer of 2016 were included.
Stools were transported rapidly to the laboratory, seeded on Karmali medium incubated at 37°C in a microaerobic atmosphere for a maximum of 72 hours, and tested with the Orion GenRead Campylobacter test. After culture, suspected colonies were identified by MALDI-TOF mass spectrometry. In addition, once a week an in-house PCR specific for *Campylobacter jejuni/coli gyr*A gene as well as an ELISA detecting Campylobacter antigens (RidaScreen Campylobacter, R-Biopharm) were carried out.

Results: One hundred and eighty patients were included during this period, and 33 (18.3%) were positive for Campylobacter (26 by culture and 7 culture negative but PCR and ELISA positive). There were 29 *C. jejuni* and 4 *C. coli*. Orion GenRead Campylobacter detected 32 of the 33 positive stools, and 4 false positives out of 147 negative stools (sensitivity 96.9%, specificity 97.2%).
A specific apparatus is required to perform this test and results can be obtained within 2 hours for 12 samples.

Conclusion: In conclusion, Orion GenRead Campylobacter has an excellent sensitivity and specificity, provides a quick result and is easy to use.

Surveillance of *Campylobacter ureolyticus* in Japan and development of multiplex PCR to detect *C. jejuni*, *C. coli* and *C. ureolyticus*

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Campylobacter ureolyticus is a potential human pathogen associated with gastroenteritis. In our previous study, *C. ureolyticus* was detected in 27% (177/667) stool specimens from children with diarrhea in Japan. Interestingly, this prevalence of *C. ureolyticus* was much higher than that of *C. jejuni* and *C. coli* which are known as leading causes of campylobacteriosis worldwide. As there were no reports about *C. ureolyticus* in diarrhea in children in Japan, to evaluate an association of *C. ureolyticus* with diarrhea in children in Japan, we conducted a case control study. Rectal swabs (n = 244) were collected from children with and without diarrhea during November 2015 to August 2016 in Okayama, Japan. *C. ureolyticus* was screened by a species-specific-PCR and -culture methods. The bacterium was detected in 36% (69/189) children with diarrhea whereas it was detected only in 11% (6/55) children without diarrhea (Odds ratio: 4.7, 95% confidence interval: 1.91-11.5). This data indicated that *C. ureolyticus* is associated with diarrhea in children in Japan. As routine culture method for *C. jejuni* and *C. coli* is not applicable to *C. ureolyticus*, it might have remained undetected in Japan so far, suggesting the importance of development of easy and reliable detection methods. Therefore, we have developed a multiplex PCR for the detection of *C. ureolyticus* including *C. jejuni* and *C. coli*. This multiplex PCR might be helpful to extensively increase epidemiological data of *C. ureolyticus*.

Recombinase Polymerase Amplification (RPA)
for the detection of *Campylobacter* in chicken faeces

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Conventional culture methods for the detection of *Campylobacter* are sensitive but take 2-5 days to complete. PCR-based methods are quicker, but require state-of-the-art laboratory facilities. Recombinase Polymerase Amplification (RPA) is an isothermal DNA amplification technology working at low temperatures (optimum at 37^{o}C). Since thermal cycling is not required, equipment is much cheaper and results can be read with a portable machine. Being a closed system, the low risk of cross contamination makes it also suitable for on-site testing.
The performance of a commercial kit, the TwistAmp® exo +Campylobacter kit (Twist Dx), was compared with qPCR (as described by Josefson et al., 2004) and culture (ISO 10272-1:2006). For both nucleic acid methods, sample material was treated minimally by suspending 1 gram of faecal material in 9ml saline. After settling of the debris, the supernatant was heated for 10 min at 95^{o}C. In each test five µl was tested. Additionally, total DNA was isolated from the supernatant using the NucliSENS® easyMAG® (bioMérieux). A total of 84 samples were tested, 30 of which were positive in culture.
RPA detected *Campylobacter* in 29 heat treated samples (26 of which were also positive in culture), whereas the qPCR only showed invalid results due to autofluorescence/inhibition. On purified DNA, both assays performed well with overall agreements with culture of 88% (qPCR) and 89% (RPA). Overall agreement between the two molecular assays was 99%.
The results indicate that RPA allows very simple sample preparation. We identified *Campylobacter* contaminated flocks within one hour with 96% sensitivity by sampling faecal material from chicken transport crates. In conclusion, RPA may be a suitable method for on-site detection of *Campylobacter* in chicken faeces, as it is not very demanding concerning the quality of input DNA, and gives rapid results that can be read with a portable machine.

Detection of *Campylobacter fetus* in faeces samples from small ruminants using real-time PCR and MALDI-TOF

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The pathogen *Campylobacter fetus* can cause acute gastroenteritis and occasionally septicaemia. It is an uncommon species of the *Campylobacter* genus, that can be distinguished from other *Campylobacter* species due to its ability to grow under microaerophilic conditions at 25 °C. Infections caused by *Campylobacter fetus* are often severe and the main source of human infections arefood products from cattle and small ruminants. Therefore the prevalence of *C. fetus* among small ruminants was monitored in this study by examining faeces samples from dairy farmsrearing sheep or goat. The samples were enriched in Lander's medium and subsequently plated on Skirrow medium for isolation. Isolated colonies were examined for growth under microaerophilic and aerobic conditions at 25 °C at 41,5 °C. Subsequently, isolates were identified by MALDI-TOF and the presence of the *nahE* gene by an in-house developed real-time PCR. The results revealed that several non-*Campylobacter fetus* isolates show growth at 25 °C, indicating that growth at 25 °C under microaerophilic conditions is not sufficient to differentiate *C. fetus* from other *Campylobacter* species. On the other hand, the developed real-time PCR detecting the *nahE* gene and the MALDI-TOF technique were sufficient to distinguish *C. fetus* isolates from other *Campylobacter* species.

For this surveillance study, a total of 184 farms rearing goat and 24 farms rearing sheep were visited, and 5 samples per farms were examined for the presence of *C. fetus*. Only 4 samples were positive for *C. fetus*, of which 3 samples originated from the same farm. This indicates that the prevalence of *C. fetus* among dairy farms rearing sheep and goat in the Netherlands is low.

Development of internal sample process control for quantification of live *Campylobacter* on broiler meat by real-time PCR

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Quantification of *Campylobacter* on broiler meat is currently based on the cultivation method according to ISO 10272-2. It provides an indication of bacterial colony forming units (CFU) that is appropriate for meat samples directly contaminated by leakage of intestinal content harboring *Campylobacter* during slaughtering. Bacterial cells might lose cultivability due to cold or oxygen stress while poultry meat is stored at retail. These cells, although potentially infectious, are not detected by culturing. An alternative is real-time PCR with a pretreatment of the samples with fluorescent dyes as propidium monoazide (PMA). Reliable result from this method can be obtained when the signal from dead cells is sufficiently reduced. This reduction is influenced by various parameters that need to be monitored by an internal sample process control (ISPC). The aim of the study was to develop the ISPC serving as a reference standard with a specified number of dead *Campylobacter* to monitor reduction of the signal from dead cells and putative DNA losses from live cells during sample processing. The target for the ISPC was selected based on literature survey, sequence analysis and verified in exclusivity studies. In total 248 isolates belonging to the Campylobacterales were studied. Further, the chicken rinse samples were inoculated with live, dead or mixture of live and dead *Campylobacter jejuni* and with the selected target for ISPC. The samples were treated with the PMA and the real-time PCR was performed. Exclusivity studies resulted in lack of false positive results. This verified the suitability of the selected target sequence as a control for detection of *C. jejuni*, *C. coli* and *C. lari.* Experiments with inoculated chicken rinse confirmed that the added ISPC detected similar variation in the signal reduction from dead cells caused by the DNA isolation and sample-specific absorption of intercalating dyes. Further research is needed to conserve the standard for distribution to various laboratories.

Evaluation of *Campylobacter* isolation methods for samples collected from poultry farms and slaughterhouses in Thailand

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Campylobacter is one of the leading causes of bacterial gastroenteritis in humans. Although a standard isolation method such as ISO 10272-1, has been routinely used to detect *Campylobacter* from food samples, this method may not be suitable for all sample types and in fact it can rarely recover *Campylobacter* from high background samples such as samples from farms and raw poultry products. The objective of this study was to identify *Campylobacter* isolation methods appropriate for samples collected from broiler farms and slaughterhouses in Thailand. Cloacal swabs, fresh feces and boot swabs were collected from 60 broiler flocks, whereas caecal, neck/breast skin and carcass rinse samples were obtained from 30 broiler batches. Isolation methods including ISO 10272-1, the United States Food Safety and Inspection Service (FSIS) protocol, OIE recommended procedure as well as *Campylobacter* isolation methods previously published in peer-reviewed journals were used to detect *Campylobacter* from different sample types. The positive rates obtained from each method were compared using chi-square test ($P < 0.05$). The results reveal that direct plating on modified Charcoal-Cefoperazone-Deoxycholate Agar (mCCDA) combined with Preston agar give the highest positive rate for *Campylobacter* isolation from cloacal swab samples ($p = 0.65$) and boot swab samples ($p = 0.002$). On the other hand, the highest recovery rate from fresh feces ($p = 0.85$) and caecal samples ($p = 0.62$) was obtained by direct plating on mCCDA alone. For neck/breast skin and carcass rinse samples, Preston broth with mCCDA provided better *Campylobacter* isolation rates than Bolton broth with mCCDA, the ISO 10272-1 recommended media for food samples, although this difference was not statistically significant ($p > 0.05$). In conclusion, the appropriate *Campylobacter* isolation methods should be considered and selected for each sample type, particularly for samples with high background microorganisms.

Selective Medium for Growth of *Campylobacter* in Containers Incubated Aerobically

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Introduction: *Campylobacter* are traditionally cultured in primary containers inside of secondary containers filled with microaerobic atmospheres. Recent findings indicated that media supplemented with optimal concentrations of amino acids, organic acids, and bicarbonate support *Campylobacter* growth without incubation in microaerobic atmospheres. The goal of the current research was to determine if supplementing this medium with antibiotics would selectively support *Campylobacter* growth in mixed bacterial cultures.

Materials and Methods: A basal medium composed of (g/l), beef extract, 50; tryptose, 10; soluble starch, 10; sodium lactate 3.0; agar, 0.5; and sodium bicarbonate, 0.15 was prepared. The medium was supplemented with no antibiotics (control), Campy-Cefex antibiotics (cycloheximide, 200 mg/l and cefoperazone, 33 mg/l), or Campy-Cefex antibiotics + trimethoprim lactate, 10 mg/l). Ten ml aliquots of media were transferred to 25 ml culture flasks and inoculated with approximately 10^3/ml of pure cultures of *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter jejuni*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes*, or *Salmonella* Kentucky or with mixed cultures of a *Campylobacter* isolate and a non-*Campylobacter* isolate. After aerobic incubation at 37C for 48 h, *Campylobacter* were enumerated on blood agar with Blaser-Wang Selective supplement incubated microaerobically for 48 h. Non-*Campylobacter* isolates were enumerated on the appropriate selective agar medium.

Results: Approximately 10^8 cfu/ml of each *Campylobacter* spp. was recovered from all media inoculated with pure *Campylobacter* cultures or mixed cultures, while approximately 10^9 cfu/ml of non-*Campylobacter* cultures were recovered from control media inoculated with pure or mixed cultures. *E. faecalis* was the only non-*Campylobacter* recovered from media supplemented with Campy-Cefex antibiotics, while no non-*Campylobacter* were recovered from media supplemented with Campy-Cefex antibiotics + trimethoprim.

Conclusion: The recently described medium supplemented with Campy-Cefex antibiotics + trimethoprim can be used to isolate *Campylobacter* from mixed bacterial cultures in primary containers incubated aerobically. Utilization of this procedure will simplify methodology for isolating *Campylobacter*.

Evaluation of *C. jejuni* strains and mCCDA batches for testing the performance of the media

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The purpose of this work was to test the performance of mCCDA (modified Charcoal-Cefoperazone-Deoxycholate Agar) using *C. jejuni* control strains and different mCCDA batches. This performance was done by quantitative testing as described in the new version of the NF EN ISO 11133.

Therefore, two *C. jejuni* strains (ATCC 29428 and ATCC 33560) were used and various mCCDA batches were tested. These mCCDA batches came from three providers and were either ready to use or prepared in our lab.

This study showed that all the tests for selectivity were fine and no difference was observed between providers.

However, results were different for the productivity tests not only between the two *C. jejuni* strains but also between mCCDA batches. Eight productivity tests out of twelve were correct for the *C. jejuni* strain ATCC 33560 whereas only three productivity tests out of twelve were correct for *C. jejuni* strain ATCC 29428. When issuing from a same provider, homemade mCCDA gave five good results to the five tests performed whereas ready to use mCCDA only lead to one good result for five tests performed.

These results show that performance testing according to the NF EN ISO 11133 can lead to unacceptable results following the *C. jejuni* strain and/or the mCCDA batch used.

Comparison of four different selective media for the quantification of *Campylobacter* in poultry meat and rapid confirmation of suspect colonies

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Quantification of *Campylobacter* in food products, especially in those products known to be highly contaminated, such as fresh poultry meat, is becoming common. Over the last years, different new selective media for the enumeration of *Campylobacter* have been developed.
The present study aimed to evaluate the performance of four commercially available selective media for the quantification of *Campylobacter* in broiler meat and a latex test kit for the identification of thermophilic *Campylobacter*.
Campylobacter counts in naturally contaminated broiler skin samples were determined using the following selective *Campylobacter* media: Modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA), CHROMagar Campylobacter (CHROM), RAPID'*Campylobacter* Agar (RAPID) and Campy Food Agar (CFA). Since all samples were only contaminated with *C. jejuni*, deep frozen broiler samples were artificially contaminated with *C. coli* and *C. lari* after defrosting, and the counts were determined using the same four selective *Campylobacter* media. Besides the enumeration of suspect *Campylobacter* colonies on all selective media, also the non-suspect *Campylobacter* colonies were enumerated. From each selective medium with countable suspect *Campylobacter* colonies, the species of up to 3 colonies was confirmed by PCR. Furthermore, thermophilic *Campylobacter* and non-*Campylobacter* collection strains able to grow on CHROM were tested using the Microgen *Campylobacter* Latex.
Comparison of *Campylobacter* counts in naturally contaminated samples with the four selective media indicated that the mean count obtained with RAPID was somewhat lower than the means obtained with the other three selective media. No difference in counts between the four media was obtained for artificially contaminated samples. Non-suspect *Campylobacter* colonies were only frequently present on mCCDA. All *Campylobacter* strains able to grow on CHROM gave a positive reaction whereas the non-*Campylobacter* strains gave a negative reaction with the Microgen *Campylobacter* Latex.

Evaluation of a new method for the enumeration of *Campylobacter* in chicken carcass rinse samples.

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Campylobacter is the major leading cause of foodborne illness in many countries worldwide due mainly to consumption of contaminated and undercooked poultry and poultry products. A new TEMPO® method for the enumeration of *Campylobacter* in 2 days without confirmation step has been developed and compared to *Campylobacter* reference method.

More than 180 samples were collected from 12 various poultry slaughter plants (mainly pre-chilled and post-chilled carcass rinses). These samples were tested by the TEMPO® *Campylobacter* method against the USDA-FSIS procedure for thermotolerant *Campylobacter* enumeration using Campy-Cefex agar.

TEMPO® *Campylobacter* method showed a strong correlation (r=0,96) compared to FSIS method with a slope close to 1 and an intercept equal to -0,23.

97,5% of the TEMPO® results were within the interval ± 0,8 log versus the FSIS method.

The TEMPO® *Campylobacter* method is an accurate, rapid and automated alternative method for thermotolerant *Campylobacter* count in 2 days, without tedious confirmation steps, in poultry samples.

Publication of revised International Standard ISO 10272 for detection and enumeration of *Campylobacter* in the food chain

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On behalf of the CEN/TAG19 working group

Revised ISO 10272-1 and ISO 10272-2 finally were published in June 2017.
ISO 10272-1:2017 specifies a horizontal method for the detection by enrichment or direct plating of *Campylobacter* spp. It cancels and replaces the first edition (ISO 10272-1:2006).
ISO 10272-2:2017 specifies a horizontal method for the enumeration of *Campylobacter* spp. It cancels and replaces ISO/TS 10272-2:2006.
The previous editions of both parts have been technically revised with the following main changes:

- Samples from the primary production stage have been added to the scope (both parts);
- The detection method was extended to include the option of a second enrichment broth (Preston broth), primarily to overcome problems with background flora resistant to third generation ß-lactams (like cefoperazone in Bolton broth) (part 1);
- The detection method was extended to include the option of direct plating on mCCDA (part 1);
- The note on the use of closed containers with reduced headspace as an alternative to incubation in a microaerobic atmosphere has been deleted (part 1);
- Serial dilutions are plated in single instead of in duplicate, to be in line with ISO-7218 (part 2);
- The confirmation tests on study of microaerobic growth at 25°C and aerobic growth at 41,5°C were replaced by the study of aerobic growth at 25°C (both parts);
- Performance testing for the quality assurance of the culture media has been added to the normative Annex on the preparation of the culture media (both parts).
- Performance characteristics (specificity, sensitivity and LOD_{50} (level of detection at 50%) as obtained from the interlaboratory validation study have been added to an informative Annex (part 1).
- Performance characteristics (repeatability standard deviation, repeatability limit, reproducibility standard deviation, reproducibility limit) as obtained from the interlaboratory validation study have been added to an informative Annex (part 2).

Development of a quantitative and qualitative chemotaxis assay, tHAP, a modified hard plug assay, using *Campylobacter jejuni* model

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The Hard Agar Plug (HAP) method is frequently used to study the chemotactic behaviour of *Campylobacter jejuni*. This assay has shortcomings of not being directly quantitative and is not suitable for the observation of chemotaxis over short time periods or investigating repellent taxis. It is also prone to give false positive and negative responses. The aim of this study to design an accurate and rapid chemotaxis assay with both qualitative and quantitative output for investigation of bactiral chemotactic responses using *C. jejuni* as a model organism. This study describes a chemotaxis assay based on a basic HAP assay (tHAP) that includes the addition of Triphenyltetrazolium chloride (TTC) to enable quantification of chemotactic responses of *C. jejuni toward*s attractants and repellents. Inclusion of TTC enables colourimetric detection of bacterial cells, actively metabolising by enzymatic reduction of TTC- to TFP- Red (1, 3, 5-Triphenylformazan), migrating toward/away from a plug containing a chemoeffector. This modification allows quantitative assessment of chemotaxis both by colourimetric measurement and by viable count over a time period between 30 minutes and 3 hours. The tHAP assay of chemotaxis demonstrated a dose-responsive chemotactic motility of *C. jejuni* cells toward a concentration gradient of the attractants, aspartate and serine. Importantly, the tHAP assay was used to differentiate between repellents and attractants by using a competitive modification of the assay. The tHAP assay is a promising method for both qualitative and quantitative assessment of bacterial chemotactic motility with respect to attractants and repellents. This version of HAP-based assay provided a rapid, accurate qualitative and quantitative assay to explore the chemotaxis of *C. jejuni*.

New colorimetric aptasensor for rapid on-site detection of *Campylobacter jejuni* and *Campylobacter coli* in chicken carcass samples

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Campylobacter is the most common cause of infectious intestinal disease, with almost all cases caused by two species: *C. jejuni* and *C. coli*. We recently reported a gold nanoparticle-based two-stage aptasensing platform, which was improved in the present study for the rapid and on-site detection of both *C. jejuni* and *C. coli* in food samples. Compared to the previous platform, the improved platform yielded a more obvious colour change from red to purple due to the aggregation of gold nanoparticles, and does not require additional time or a pH optimization step for the aptamers to be adsorbed onto the gold nanoparticles. Using a highly specific aptamer that binds to live *C. jejuni* and *C. coli*, the improved aptasensor worked perfectly with all pure culture samples. The accuracy of the newly developed platform was comparable (p = 0.688) to that of the gold-standard detection method of tazobactam-supplemented culture, whereas it was superior to the official agar-based detection method (p = 0.016) in a validation study with 50 naturally contaminated chicken carcass samples. This is the first study on a colorimetric sensor that targets both live *C. coli* and *C. jejuni* in naturally contaminated samples. In addition, we provide the first evidence that both morphological status and the amount of *Campylobacter* present play key roles in the effectiveness of colorimetric detection. Thus, suitable selection of an antibody or aptamer with consideration of the morphological status of pathogens in samples is essential for direct detection without enrichment. Our data suggest that application of the developed sensor could provide an excellent screening method with a reduction in the detection time from 48 h to 30 min after enrichment, thus saving time, labour, and cost.

Violet-blue light as a novel probe to reveal mechanisms of oxidative damage in *Campylobacter jejuni*

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Campylobacter jejuni is an oxygen-sensitive microaerophile which may be susceptible to the effects of reactive oxygen species (ROS) due to possession of ROS sensitive enzymes essential for metabolism. Here, we propose 405nm violet-blue (VB) light as a new tool to study the response of *C. jejuni* to ROS. The bactericidal effects of VB-light have been extensively investigated in taxonomically diverse bacterial pathogens, however the exact mechanism of killing remains poorly defined. Previous research has suggested that VB-light induces ROS release through activation of endogenous porphyrin molecules. Using high intensity 405nm light generated from a light-emitting diode (LED), we have confirmed that *Campylobacter jejuni* shows higher sensitivity to killing by VB-light compared to other Gram-negative pathogens. This may be due to increased light absorption, more susceptible ROS targets or both. Interestingly, absorption spectra of intact cells of *C. jejuni* shows a strong peak at 405nm, suggesting an abundant haem/porphyrin species as the light absorber , but since this peak remains in a cj1153 mutant which is devoid of periplasmic c-type cytochromes, it must represent another porphyrin species. We show that irradiation with 405 nm light causes release of ROS in *C. jejuni*, as measured using the fluorescent reporter dihydrodichlorofluorescein. Through comparisons of (i) ROS production and (ii) cell viability over a range of doses of VB-light in the wild-type and isogenic deletion mutants in a variety of oxidative stress protection enzymes, we have evaluated for the first time which systems are most important in the response to ROS generated *within* the cell. VB-light is thus a novel and more precise tool for studying oxidative stress in *Campylobacter* through induction of ROS intracellularly as opposed to conventional external treatment using chemicals like hydrogen peroxide or paraquat, which have undesirable side-effects.

Development and Application of Oxidative Stress Sensing and Response Fluorescent Proteins in *Campylobacter jejuni*

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As a microaerophilic organism, *C. jejuni* actively defends against oxidative stress encountered both in the host and in the environment. How *Campylobacter* responds to these toxic stresses in real time remains poorly understood. In this study, we developed and expressed redox-sensitive GFP proteins in *C. jejuni* to measure in real time intracellular redox balance changes.

Two previously characterized redox-sensitive GFP proteins RoGFP2 and Hyper3 were utilized to construct two oxidative stress sensors in *Campylobacter*, including $RoGFP2_{cj}$ and $Hyper3_{cj}$. The synthesized genes were cloned into *C. jejuni* isolate IA3902. Expression of the oxidative stress sensor proteins in *C. jejuni* was confirmed by Western blotting and confocal fluorescence microscope (CFM). The *C. jejuni* isolates carrying the sensor proteins were further evaluated for responses to exogenously added H_2O_2 and different classes of bactericidal antibiotics.

The results showed that the *C. jejuni* isolates carrying $RoGFP2_{cj}$ or $Hyper3_{cj}$ generated detectable level of fluorescence when measured by a microplate reader. Although the $RoGFP2_{cj}$ construct is highly fluorescent, it did not respond to treatment with H_2O_2 *in vitro*. In contrast, the $Hyper3_{cj}$ construct generated a robust change in fluorescent signals upon treatment with 50 μM H_2O_2, indicating that $Hyper3_{cj}$ is responsive and effective to detect oxidative stress in *Campylobacter*. Treatment of the $Hyper3_{cj}$-containing isolates with bactericidal antibiotics including ciprofloxacin, kanamycin and ampicillin did not trigger changes in fluorescence, suggesting these antibiotics do not generate oxidative stress *in Campylobacter*. Under CFM, the $RoGFP2_{cj}$ construct was much more fluorescent than the $Hyper3_{cj}$ construct, and *C. jejuni* IA3902 cells harboring $RoGFP2_{cj}$ were strongly fluorescent when co-cultured with sheep trophoblast cells, indicating $RoGFP2_{cj}$ is suitable for tracking *C. jejuni* interaction with cells.

These results demonstrate the feasibility of real-time measurement of intracellular redox balance in a microaerophilic organism and the usefulness of these fluorescent constructs for understanding *Campylobacter*-host/environment interactions.

Can a visual loop-mediated isothermal amplification assay stand out in different detection methods when monitoring *Campylobacter jejuni* from diverse sources of samples?

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Consumption and exposure to *Campylobacter*-contaminated food are important causes of bacterial diarrhea. This paper reports the development and evaluation of a visual loop-mediated isothermal amplification (LAMP) assay targeting the highly conserved gene of *Campylobacter jejuni* (*C. jejuni*). This assay could be used for specialized detection and primary screening of retail food samples in China. When applied to 475 different samples, PCR (14.9%) and bacterial culture (12.7%) showed lower detection rates than the LAMP, which demonstrated 51 positive (16.6%) with a 100% diagnostic sensitivity to bacterial culture with no targets undetected. When tested with samples that had different cleanliness, the specificity (0.955), accuracy (0.961), positive likelihood ratio (22.417), kappa coefficient (0.844) for retail food samples tested by LAMP were higher than for fecal and environmental samples. Moreover, when tested with food samples that had different *C. jejuni* carry rates, LAMP was more sensitive in monitoring raw pork meat samples- the specificity (0.958), accuracy (0.958), and positive likelihood ratio (23.600) were higher than for chicken samples compared with a bacterial culture. The LAMP test enables the cost-effective detection of *C. jejuni* in food samples in 40 min, and it can also function as a primary screening approach or a complementary method in large-scale and on-site assays of food.

Comparison of liquid growth medias for cultivation of *Campylobacter concisus*

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Introduction: Emerging *Campylobacter concisus* is an oral bacterium associated to prolonged gastroenteritis and inflammatory bowel disease (IBD). *C. concisus* requires H_2-enriched microaerobic conditions for growth. Studies suggest that the supplementation of defibrinated horse blood (DHB) to liquid growth medias (LGM) can aid growth, however comparison of different LGM has been sparsely investigated.

Material and methods: Clinical *C. concisus* isolates from nine patients (IBD n=4, healthy controls n=3, gastroenteritis n=2) in different locations (saliva (n=2), faeces (n=3) and mucosal biopsies (n=4)), were tested for growth in LGM. Bacterial cell suspensions (McFarland standard 0.5 equivalent to 1.5 $*10^8$ CFU/ml) were added to four LGM broths (Brain Heart Infusion (BHI), Bolton (BOL), Mueller Hinton (MH) and Tryptase Phosphate (TP)) with and without added DHB (5%). At baseline, and 24 hours after incubation at 37°Cin a microaerobic atmosphere (80% N_2, 10% CO_2, 5%H_2, 5%O_2), the media was diluted to $1.5*10^2$ CFU/ml and 100 µL suspension was streaked on 5% blood agar plates with added yeast. Plates were subsequently incubated in microaerobic conditions for 48hours and CFU'swere counted successively. All experiments were performed in triplicate.

Results: *Campylobacter concisus* was able to grow following incubation in all tested LGM. The lowest growth rate for all isolates was observed in MH broth without DHB (p=0.005). There was no significant difference between growth rates in all other tested LGM (p=0.226). Supplementation of DHB did not aid growth for any media, but inhibited growth in TP broth (p=0.027).

Conclusion: All tested LGM can be used in the cultivation of *C. concisus,* and could possibly aid in cultivation of fastidious *C. concisus* from clinical samples. Supplementation of DHB did not enhance growth rates, when added to any tested media.

Rapid and specific methods to detect *Campylobacter hepaticus*, causing spotty liver disease in chickens, and differentiate from other human *Campylobacter*

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Spotty liver disease (SLD) causes significant egg production losses and mortality in chickens. It was first reported in the 1950s and there have been sporadic reports of the disease throughout the intervening decades. Recently it has become of increasing concern in Australia and the UK as outbreaks of the disease have occurred more frequently. The organism that causes the disease was recently identified, isolated, and characterised as a new bacterial species, *Campylobacter hepaticus*, by our group. In this study, a specific and sensitive quantitative real-time PCR assay, based on the glycerol kinase gene from *C. hepaticus,* was developed to facilitate the epidemiological investigations of *C. hepaticus* in tissue samples from clinical cases of SLD. The assay could detect as few as 10 organisms. It was applied to samples taken from liver, bile, small intestine and caecum, and also to cloacal swabs. The qPCR assay of 45 birds from eight SLD outbreaks showed that *C. hepaticus* colonisation level in these birds ranged from 1.7×10^4 CFU/g to 1.6×10^9 CFU/g in their tissues. Quantitative PCR analysis of samples from the gastrointestinal tract mucosa of artificially infected birds showed that *C. hepaticus* numbers increased from duodenum to jejunum to ileum. The ability to reliably quantify *C. hepaticus* in cloacal swabs is particularly beneficial, as it provides a relatively non-invasive method to detect infected birds for epidemiological investigation of SLD in the field. In addition, we have developed a multiplex PCR that enable the co-detection of *C. hepaticus*, *C. coli* and *C. jejuni* from chicken tissue samples using new targeted primers for *C. jejuni* and *C. coli* as some commonly used primers also cross react with *C. hepaticus*. The multiplex PCR assay is useful for epidemiological investigations of *C. hepaticus* and detection of important *Campylobacter* human pathogens within chicken and environmental samples.

Gastric juice-based real-time PCR for tailored *Helicobacter pylori* treatment: A practical approach

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Aim: A gastric juice-based real-time PCR assay was established to identify *H. pylori* infection, clarithromycin susceptibility and human CYP2C19 genotypes and to guide the tailored *H. pylori* eradication therapy.

Methods: From January 2013 to November 2014, 178 consecutive dyspeptic patients were enrolled for the collection of gastric biopsy samples and gastric juice by endoscopy, including 105 *H. pylori*-positive patients and 73 *H. pylori*-negative patients in this study. A series of primers and probes were distributed into four reactions dedicated to the *H. pylori cagH* gene coupled with an internal control (the *Rnase P* gene), the A2142G and A2143G mutants of the *H. pylori 23S rRNA* gene, SNP G681A of *CYP2C19*2* and SNP G636A of *CYP2C19*3*. The E-test and DNA sequencing were used to identify the *H. pylori* clarithromycin susceptibility phenotype and genotype. The *CYP2C19*2* and *CYP2C19*3* SNPs were also evaluated by nucleotide sequencing. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of this gastric juice-based real-time PCR assay were evaluated by comparison with the same measures obtained through gastric biopsy-based PCR and culture.

Results: The *H. pylori* diagnostic sensitivities of the culture, PCR, and gastric biopsy- and gastric juice-based real-time PCR assays were 90.48%, 92.38%, 97.14%and 100%, respectively, whereas the specificities of the above methods were all 100%. Higher false negative rates were found in the gastric biopsy samples assessed by culture (10.48%), PCR (7.62%) and real-time PCR (2.86%) than were found in gastric juice by real-time PCR. Regarding clarithromycin susceptibility, a concordance of 82.98% and discordance of 17.02% were observed among these different methods. These discrepancies mainly represented the difference between the *H. pylori* clarithromycin susceptibility phenotype and genotype.

Conclusions: This gastric juice-based real-time PCR assay is a more accurate method for the detection of *H. pylori* infection, clarithromycin susceptibility and *CYP2C19* polymorphisms.

Detection of *H. pylori* in gastric biopsy material

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The current method in use at the Helicobacter Reference Service for the detection of *H. pylori* is culture of biopsy specimens. As *H. pylori* is a fastidious, microaerophilic organism, there are various confounding factors for the detection of *H. pylori* including: the length of time between collection and receipt of the specimen, contamination of the biopsy at the point of collection and the microaerophilic conditions used that can limit the success of culture based techniques.
From January-December 2016, the Helicobacter Reference Service received 1113 biopsy specimens. *H. pylori* was successfully cultured from 383 specimens using 10% Columbia Blood Agar and incubation under microaerophilic conditions (37ºC +/- 1ºC, 5% O_2, 10% CO_2, 5% H_2 and 80% N_2), in a Don Whitley Modular atmosphere-controlled system (MACS-VA500). Identification was through biochemical testing and Gram staining. Phenotypic sensitivity testing to various antimicrobial agents was carried out using E-tests following BSAC guidelines.
A novel Real-Time PCR for the detection of *H. pylori* based on the *UreA* gene was developed and evaluated using a panel of 40 culture positive and 40 culture negative biopsy specimens. Of these, 35 of the culture positive specimens and 9 of the culture negative were successfully detected by this PCR. Two automated DNA extraction platforms (QiaSymphony and MagNa Pure Compact) and one manual extraction kit (DNeasy) were evaluated to compare which gave the highest DNA yield directly from biopsy material.
Future work: comparison of in house *UreA* PCR and phenotypic sensitivity testing against the Amplidiag® *H. pylori*+ClariR Multiplex real-time PCR kit and data generated through whole genome sequencing.

Poster session
« Adaptation of *Campylobacter sp.*
to environmental conditions »

Adaptive response to environmental conditions: the case of an atypical aero-tolerant *Campylobacter jejuni* strain

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As an obligate microaerophilic organism, *Campylobacter jejuni* has to survive oxidative stress throughout the food chain. Adaptive strategies to survive atmospheric oxygen during transmission to humans remain unclear for *C. jejuni*. The clinical *C. jejuni* Bf strain was characterized by an atypical habituation to O_2 atmospheric concentration. Growth in aerobic conditions (AC) was obtained in batch and on plates only for Bf strain unlike for other *C. jejuni* strains tested. A better survival under superoxide and peroxide stresses was also observed. During multiplication in AC, *C. jejuni* Bf cells display coccoïd shape with a correlated lower transcription of *mreB*, a gene involved in the maintenance of the bacillary shape in microaerobic conditions (MAC). Proteomics analyses pinpointed 47 proteins with a significant abundance difference between cells grown under MAC and AC. The higher abundant proteins during growth under AC are mainly involved in the oxidative stress response, tricarboxylic acid (TCA) cycle, iron uptake, regulation and amino acid uptake. Transcriptional and enzymatic analyses of the encoding genes involved ROS scavenging were correlated to higher transcript levels and higher catalase-equivalent activities for cells cultivated or acclimated to AC as compared to MAC. Taken together, these data revealed that the atypical aerotolerance of Bf could be correlated to a greater ability to scavenge ROS and to maintain a functional TCA cycle. This ability could explain the emergence of *C. jejuni* in various environmental conditions.

Identification of genes associated with environmental resistance in *Campylobacter jejuni* isolates from processing in a broiler slaughterhouse

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Campylobacteriosis is nowadays the main food-borne zoonosis around the world. The causing agents are several species of *Campylobacter* gender, being *C. jejuni,* which main reservoir is chicken, the most frequent species involved in human cases. We have determined the prevalence of htrB, htrA and ppk1 genes in DNA isolates of *C. jejuni* obtained in a previous study. These genes are involved in the environmental resistance of *C. jejuni.* Isolates used in this study were obtained from 1) broilers on arrival at the slaughterhouse (initial stage of processing; 31 DNA samples) and 2) from chicken meat ready to be consumed (final stage of processing; 24 DNA samples). PCR was used to identify the presence of each gene. The existence of significant differences between the prevalence of both stages for every gene was evaluated.

A global prevalence of 87.3%, 27.3% and 85.5% for genes htrB, htrA and ppk1, respectively, was observed. With a 95% of confidence level, significant increases from initial to final stage were observed for htrB and htrA genes. It could be related to a selection of *C. jejuni* isolates in the final stage according the presence of these genes. Our results support empirically the implication of the studied genes in the environmental resistance of *Campylobacter jejuni.*

To give and take:
Campylobacter spp. and the microbiome

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Bacteria found on food, including food-borne pathogens, have to survive in different, often stressful environments. Most of these bacteria have a broad range of different genes, gene-controlling and expression systems to react to these environmental stressors. For *Campylobacter*, a fastidious microaerobic bacterium, with a rather small genome, without this broad range of tools, this is a specific challenge. As *Campylobacter* regularly encounters many different microorganisms, e.g. *Firmicutes* like *Lactobacillales*, *Bacillales*, and *Clostridiales*; *Enterobacteriales* like *Escherichia coli*; and *Pseudomonadales* it benefits from but can also support in specific bacterial-bacterial interactions. Here we show that *Campylobacter* can on the one hand side support the growth of strictly anaerobic *Clostridia* spp. under microaerobic conditions within a naturally formed mixed biofilm and on the other hand is able to use *Pseudomonas* spp. to survive ambient oxygen levels also under refrigeration temperature of 3°C.

The study was undertaken with different *Campylobacter* spp. food isolates and type stains as well as *Clostridium and Pseudomonas* type strains and food isolate. To characterize interactions between *Campylobacter* spp. and *Clostridia* spp. incubation was performed *in vitro* in micro-well plates under microaerobic conditions, which were advantageous for the growth of thermophilic *Campylobacter* spp. but do not allow the strictly anaerobic *Clostridium* spp. to grow. Incubation of mixed cultures (*Campylobacter* and *Clostridium*) allowed growth of both species within a biofilm that was not assembled in single-species wells.

When incubating *Campylobacter* spp. with *Pseudomonas* spp. (except *Ps. aeruginosa*) under aerobic conditions at a temperature of 3°C not favoured by *Campylobacter*, *Campylobacter* cells could be reanimated or even started to grow powered by the growth of *Pseudomonas* spp. under these conditions. When *Pseudomonas* starts to grow at 3°C *Campylobacter* cells seem to recover after a lag phase of 20 hours.

We hypothesize that the interaction between *Campylobacter* and other bacteria accounts for the small genome of *Campylobacter* to compete with environmental stress and that this interactions display the game of give and take in the microbial community.

Persistence of *Campylobacter jejuni* in raw milk – insights from whole-genome sequencing

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In November 2012, a campylobacteriosis outbreak originated from a dairy farm, and identical *Campylobacter jejuni* isolates, as revealed by pulsed-field gel electrophoresis, were recovered from the patients, cattle, and bulk tank milk. The outbreak strain persisted in the milk for a six-month follow-up, even though hygienic measures were implemented on the farm. Meanwhile, also five other *C. jejuni* types were isolated from the cattle, but none of them were detected in the milk. Further, the outbreak strain survived in refrigerated raw milk for four days, whereas the other isolates survived less than two days. The outbreak strain formed biofilm on a polystyrene plate in quantities matching the control strain (NCTC11168) and one bovine isolate.

To investigate genomic features behind the persistent phenotype of the outbreak strain, the farm isolates were subjected to whole-genome sequencing. The reads were assembled *de novo* and the contigs annotated in RAST. Compared with the other isolates, the outbreak strain harbored unique annotated genes related to apparently active pathways: methylglyoxal metabolism and capsular heptose biosynthesis. Methylglyoxal production facilitates environmental adaptation, but its accumulation may cause cell death and thus, needs to be controlled by detoxification. Capsular polysaccharides play a role in environmental persistence and survival, but also in pathogenesis.

In addition, both the outbreak strain and the other biofilm-forming isolate appeared to have functional conjugative transfer and type IV secretion system (T4SS), encoded in pVir plasmid. DNA exchange via conjugative T4SS increases genomic plasticity, thus aiding adaptation to environmental changes. Along with T4SS, pVir plasmids carry genes related to host invasion and virulence.

This preliminary study revealed genomic features that differentiated the outbreak strain from the other, non-persistent farm isolates and potentially played a role in environmental survival and pathogenesis. However, more research is needed to verify any causal linkage between the genotype and phenotype.

Dynamics, architecture and matrix of biofilms are different according to strains of *Campylobacter jejuni*

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Introduction: *Campylobacter jejuni* has been reported as the leading cause of bacterial foodborne infections in developed countries, with significant increase over the past years. Despite the fastidious growth requirements, *C. jejuni* is able to survive in the environment without permanent loss of viability and virulence. The mechanisms responsible for its survival remain unknown, but one of the survival strategies might be linked to the biofilm formation. This work was focused on detailed characterization of dynamics, spatial organization and matrix composition of *C. jejuni* biofilms, including the role of oxygen in the biofilm formation process.

Material and Methods: The objectives were achieved by analysing biofilms of two characterized strains using confocal laser scanning microscopy, transmission electron microscopy (TEM), and fluorescent lectin binding analysis (FLBA) performed with 73 different lectins.

Results: Both strains of *C. jejuni* were able to form biofilms within 17 h of cultivation. Biofilm architecture differed between the two strains, ranging from finger-like structure with voids and channels to compact multilayer-like structure that could be circumvented by a higher expression of *CosR*. Exposure of cells to oxygen enriched conditions enhanced biofilm development. FLBA screening revealed strain-specific patterns with only 6 lectins interacting with biofilm matrix of both strains. Interestingly, the biofilm matrix contained fucose that was not previously detected within *C. jejuni*. Thioflavin T and curcumin assay paired with TEM highlighted the presence of amyloids in cell envelope without association with specific cell appendages.

Conclusion: Taken together, these data provide new insights on structuralization, maturation and composition of *C. jejuni* biofilms under microaerobic and aerobic atmosphere.

wgMLST and *in vitro* study of biofilm formation in two *Campylobacter jejuni* populations isolated from a poultry slaughterhouse environment

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Introduction: *Campylobacter* biofilm formation is considered as one of the most important strategies for bacterial survival in harsh environmental conditions. Therefore, the aims of the study were i) to study genetically two *C. jejuni* populations isolated in a poultry plant environment during 17 and 21 days consecutively; (ii) to determinate the ability of these populations to form biofilm in different conditions.

Material and Methods: Ad hoc wgMLST analysis was performed in populations PFGE C (ST-904; ST-607 CC) recovered during 17 days and PFGE G (ST-3796; ST-21 CC) recovered during 21 days using Genome profile (GeP; Zhang *et al*., 2015). Moreover, biofilm formation was tested in stainless steel and polystyrene materials, different temperatures (25º C, 30º C, 37° C) and different atmospheres conditions (aerobic and microaerobically).

Results: Isolates of ST-904 showed 5 to 14 total allele differences over 1,836 shared loci (98.8% of the total genes). Similarly, isolates of ST-3796 showed 3 to 17 allele differences over 1,714 shared loci (99.1% of the total genes). wgMLST results show these populations are highly clonal lineage. Only ST-904 was able to produce biofilms at 37 ºC and 30 ºC in microaerophilic and aerobic in both type of materials. The other population, was be able to survive at 37ºC, 30ºC, 25ºC in both atmospheres and materials, but did not form biofilms.

Conclusion: The highly clonal ST- 904 population might produce biofilms in the aerobic atmosphere and stainless steel equipment of the slaughterhouse, overcoming harsh conditions. However, ST-3796 population might survive in these pre-existing biofilms. Organic matter residues must be avoided during cleaning and disinfection because they could increase the ability of *C. jejuni* to survive or form biofilms and, therefore, persist in the environment, produce cross-contamination with *C. jejuni* free batches and be spread throughout food chain.

Survival variability of *Campylobacter jejuni* strains subjected to stressful conditions

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Campylobacter is a foodborne pathogen highly prevalent in poultry and the primary cause of bacterial enteritis in humans. Stress survival of *C. jejuni* is known to vary in a strain-dependent manner, and could be explained by the wide phenotypic and genotypic diversity within its population. To ensure consumer safety, having a better understanding of strain variability enables to predict more accurately their behaviour and then potentially limit their survival. The purpose of this study was to quantify the survival variability of *Campylobacter jejuni* strains subjected to stressful conditions, using phenotypic characteristic. In addition, MultiLocus Sequence Typing (MLST) was used to identify potential correlation between survival variability and genotype.

Ten strains were subjected to the following four different stress conditions: chilled storage (14 days at 4°C), heat (5 min at 55°C), ambient temperature (48 h), and finally, cold and acid (24 h at 4°C, pH 4.3). All stresses were applied under aerobic conditions. First of all, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were performed to identify groups of strains with homogeneous behaviour. Group significance was confirmed by MANOVA. Next, intra- and inter-variability were quantified. All analyses were performed using R packages.

Three strain clusters were identified. Among studied conditions, only those associated with ambient temperature and chilled storage showed a difference between clusters: inter-variability was significantly higher than intra variability. In contrast, cold-acid and heat stress did not appear to be discriminant. The MLST revealed that genotypic and phenotypic profiles did not match: strain variability could not be associated with Sequence Type (ST). Results of this study emphasize that strain variability is essential and then has to be taken into account in exposure and risk assessment.

Thermotolerant *Campylobacter* spp: detection and enumeration during anaerobic digestion of livestock effluents in 5 biogas plants

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In the current context of developing renewable energies and recovering organic waste, on-farm anaerobic digestion represents a major challenge for the agricultural sector (energy and organic recovery of livestock manure and agricultural substrates). In France, most of biogas plants fed with manure operate at mesophilic conditions (35-40 °C) converting organic matter to biogas and by product degradation, i.e. digestate. This digestate, used as a fertilizer, is usually spread directly on land after storage in a pit. It is therefore essential to ensure the sanitary quality of digestates in order to minimize the risk of dissemination of pathogenic microorganisms during the spreading without adversely affecting the energy efficiency of the installation.
Hence, we investigated the presence of thermotolerant *Campylobacter* spp. in inputs and digestates of five biogas plants localized in Brittany, France. Three replicates of the inputs and digestates were collected at each biogas plant for the detection of *Campylobacter* after enrichment in Preston broth. *Campylobacter* was present in all the inputs with a mean concentration level varying from 97 cfu/g to 254 cfu/g. Only two digestates were found positive in *Campylobacter* at a mean concentration level of 10 cfu/g for the first and 255 cfu/g for the second. These preliminary results showed that *Campylobacter*s were able to survive after this anaerobic treatment. Therefore, further investigations are being carried out to enhance our knowledge on the becoming of *Campylobacters* during such treatments. This could help determining operational strategies for biogas plant operators to efficiently digest livestock manure and reduce consequent spreading of pathogenic bacteria in the environment.

The ovine gallbladder:
a protected niche for *Campylobacter jejuni*?

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Persistence of *Campylobacter jejuni* within flocks is of major concern from both a zoonotic health risk and as a risk for abortion in small ruminants. The recent emergence of the *C. jejuni* sheep abortion (SA) clone, represented by the isolate IA3902, within the United States over the last several decades to become the predominant isolate of *Campylobacter* identified in sheep abortion outbreaks suggests that this strain not only is able to cause disease but is able to maintain itself within the sheep population. Abattoir studies have frequently identified the gallbladder as a site of positive culture for *C. jejuni* despite the assumed inhospitable nature of this bile-rich environment. The goal of this study was to determine if previously identified putative growth factors for *C. jejuni* were located in the ovine gallbladder and to assess the location of infection within the gallbladder by *C. jejuni*. Sheep gallbladders were directly inoculated with *C. jejuni* IA 3902 and following incubation samples were collected for histopathology, histochemistry, immunohistochemistry, and scanning electron microscopy. The results of this study indicate that putative growth factors for *C. jejuni* such as neutral mucins, acid mucins, and l-fucose are present within the deep glands and on the mucosal surface of the ovine gallbladder. Immunohistochemical identification of *C. jejuni* also within the deep mucosal glands indicates that this location may play an important role in providing a protected niche within the harsh gallbladder environment for *C. jejuni* survival and long-term carriage.

Copper management and transportation in *Campylobacter jejuni*

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C. jejuni requires copper (Cu) as a cofactor for several metalloproteins, including cytochrome c oxidase. Therefore, Cu transportation and assembly is crucial for respiration, growth and host colonisation. However, copper is also being used as a growth supplement for poultry feed due to its antimicrobial effects, but how *C. jejuni* responds to excess copper is largely unknown. Excess Cu is toxic as it can activate O_2 via the Fenton reaction to generate dangerous reactive oxygen radicals. Therefore, *C. jejuni* must precisely control Cu acquisition, trafficking and incorporation into the target proteins. We have previously identified Cj1516 (CueO) as a periplasmic copper defence protein. In *C. jejuni* NCTC 11168 we now show that a cluster of genes (*cj1161c-cj1166c)* encode proteins needed for Cu homeostasis. Cj1161 is a CopA homologue and Cj1162 is homologous to CopZ. The precise roles of proteins encoded by the other genes are unknown; Cj1163 is a transmembrane protein that may have a Cu translocating function and Cj1164 is a cytoplasmic protein that has Cu binding sites. We have constructed deletion mutants in each of these genes and assessed copper sensitivity of growth, cytochrome c oxidase activity, cellular Cu content and the effect of Cu on mRNA expression using RT-PCR. The data support a model for Cu (I) efflux to the periplasm where oxidation via CueO to the less toxic Cu(II) can occur. Our results give insight into the mechanisms for both copper acquisition and tolerance in this pathogen.

Poster session

« Poultry and non-poultry epidemiology and ecology of *Campylobacter sp*. »

The population structure of *Campylobacter* colonising broiler breeder flocks

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Introduction: The study aim was to investigate the population structure of *Campylobacter* colonising broiler breeder flocks. Understanding the epidemiology of *Campylobacter* in the commercial poultry industry setting will help to develop better targeted on-farm interventions.

Materials and methods: Ten broiler breeder flocks (9 housed and 1 free-range) were sampled for *Campylobacter*. Seven of the flocks were sampled at a single time point and three were sampled longitudinally over 14 to 63 weeks. Direct culture of faecal samples, boot socks or cloacal swabs collected from the flocks gave a total of 3742 *Campylobacter* isolates. The genetic diversity of the isolates was assessed using 7 locus multi-locus sequence typing (MLST) or nucleotide sequence of the short variable region of *porA* encoding for the major outer-membrane protein. A subset of isolates was selected for whole genome sequencing (WGS), with downstream analyses based on a hierarchal gene-by-gene approach enabled by the https://pubmlst/org/campylobacter/ database.

Results: All the broiler breeder flocks were colonised by *Campylobacter*, with most having 60-90% prevalence. Between 1 and 8 *Campylobacter* strains were isolated from the flocks sampled at a single time point, and between 11 and 31 *Campylobacter* strains were isolated in total from the flocks sampled longitudinally. Some *Campylobacter* strains were able to persist over many weeks; in one example, only 1/1343 core genome loci varied within a strain colonising a flock over 3 months, and in birds tested from different houses on the same farm. The relative proportions of strains however, were found to vary over time and between different houses on the same farm.

Conclusions: Despite the most stringent levels of biosecurity applied to broiler breeder flocks, they are persistently colonised by *Campylobacter* at high levels. They represent a reservoir of strains that could potentially be spread by direct or indirect means around the industry.

Prevalence and genetic characterization of *Campylobacter* spp. in poultry farms in the North of Spain during two season periods

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Introduction: Campylobacteriosis is the leading foodborne bacterial gastroenteritis worldwide and *Campylobacter* is frequently isolated from chickens. However, the pathways by which poultry flocks acquire *Campylobacter* are not yet fully understood. Therefore, the aim was to determinate the prevalence and diversity of *Campylobacter* spp. isolated in poultry farms during autumn and spring seasons.

Material and Methods: A total of 1 188 samples were analyzed from 14 poultry farms in both seasons. Different sampling locations were analyzed: feeders, drinkers, nipple´s tray, water supply and cloacal samples from broilers. Multiplex-PCR was used to identify *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis* and *C. fetus* subsp*. fetus. C. jejuni* and *C. coli* isolates were typed by PFGE according to the method of PulseNet using the restriction enzymes *Sma*I and *Kpn*I.

Results: During autumn 42.9% of the farms analyzed were *Campylobacter* spp. positive whereas a 30.8 % was obtained during spring. Very high within-flocks prevalence was observed (43.1 % to 88.6%), being *C. jejuni* the most prevalent species. The most contaminated sample was the cloaca, followed by feeders and nipple´s tray. However, water sources were only *Campylobacter* positive in one farm. Three hundred and nine *Campylobacter* isolates were clustered into 21 PFGE types: 19 types within *C. jejuni* and 2 types within *C. coli.* Among these, 10 were identified in autumn and 11 in spring.

Conclusion: Water supply has been highlighted as a possible source of flock contamination. Moreover, the high diversity of genotypes obtained among farms suggests that environment might be one of the main sources of chicken infection. Thus, proper cleaning and disinfection procedures must be designed and applied in farms to avoid cross contamination between flocks. In that sense, *Campylobacter*-free meat could be achieved by controlling *Campylobacter* in primary production.

Occurrence and genotypes of *Campylobacter* species in broilers during the rearing period

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Poultry is the main source of *Campylobacter* infection worldwide. To obtain information on *Campylobacter*-infected flocks and create a reference for preventing and controlling *Campylobacter* at the farm level, *Campylobacter* isolates were recovered from broilers, and the environments of 9 chicken flocks in 2 farms during their growth process were observed. The genetic relationship between the *Campylobacter* isolates was determined using multilocus sequence typing (MLST). The flocks were colonized as early as 3 weeks after introduction to the farm. The highest colonization rate was more than 90%, which occurred 4–6 weeks after introduction to the farm. Quantitative data showed that the highest *Campylobacter* loads appeared at 1–2 weeks after initial colonization. *Campylobacter* loads in cloacal swabs in 4 flocks were significantly higher at 5 weeks than at 3 weeks ($P<0.05$). MLST of 171 selected *Campylobacter* isolates revealed 20 sequence types (STs), which consisted of 12 STs for *Campylobacter jejuni* and 8 for *Campylobacter coli* isolates. The STs of the *Campylobacter* isolates recovered from farm 1 were more diversified than those from farm 2. The STs of the environmental samples were highly consistent with those of the cloacal swab samples. The consistency between *Campylobacter* STs in the environmental and cloacal swab samples suggested that the environment might be one of the main sources of chicken infection. Thus, our study highlights the prevalence and contamination load of *Campylobacter* in broilers during their rearing period and emphasizes the need for control and prevention measures for *Campylobacter* infection in broilers, which is also important for human health.

Characterization of *C. jejuni* and *C. coli* broiler isolates by whole genome sequencing

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Campylobacter is the most commonly reported cause of bacterial diarrhoeal disease in humans in the EU since 2005. Most broiler batches at slaughter are colonized with *Campylobacter* and the major source of infection is contaminated poultry meat. The two species responsible for the majority of infections are *C. jejuni* and *C. coli*. The aim of this study was to use whole genome sequencing (WGS) to characterize a selection of *C. jejuni* and *C. coli* isolates from broilers. A total of 16 isolates (*C. jejuni* = 12 and *C. coli* = 4) from five broiler farms from Catalonia (northeastern Spain) were analyzed. Genomic DNA was extracted from each of the isolates and subjected to paired-end sequencing using a MiSeq platform (Illumina). The trimmed- files were *de novo* assembled using a pipeline based on Velvet algorithms. The SNP discovery on the assembled genomes was done using the pipeline available at the Center for Genomic Epidemiology (DTU, www.genomicepidemiology.org), while the identification of resistance and virulence genes was done with ResFinder version 2.1 and MyDbFinder version 1.1, respectively.
A phylogenetic analysis based on 8420 SNPs showed two main clusters grouping strains by species. All the strains showed a multidrug resistant profile, with resistance to quinolones (100%), tetracycline (81%), streptomycin (75%), erytromycin (56%) and gentamicin (13%); the main mechanisms of resistance were identified. All *C. jejuni* and *C. coli* isolates were positive for most of the 34 virulence-associated genes studied related to motility, chemotaxis, adhesion and invasion. Interestingly, the *wlaN* gene involved in the Guillain-Barré syndrome was found in two isolates. The results underline the power of WGS for investigation of virulence, clonality and antimicrobial resistance in *Campylobacter*.

On-farm risk factors associated with *Campylobacter* prevalence in conventional broiler flocks in the United States

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Campylobacter (predominantly *C. jejuni* and to a lesser extent *C. coli*) is highly prevalent in commercial poultry production and contaminated poultry meat is the most common source of infection for human campylobacteriosis, a leading cause of bacterial foodborne illness worldwide. Therefore, reduction or elimination of *Campylobacter* in poultry production has the potential impact on food safety and public health. In a recent longitudinal study, we surveyed 461 indoor conventional broiler chicken flocks reared in 53 houses/15 farms for up to 10 consecutive production cycles for *Campylobacter* presence. It was found that *Campylobacter* prevalence varied remarkably among farms, houses and flocks examined, with some houses/farms testing consistently negative while others being always positive over the entire sampling period. In the current study, a hypothesis-generating analysis using the *Campylobacter* prevalence data from the aforementioned flocks and 46 explanatory variables related to farm/house/flock-level specific standards, procedures, environmental conditions and management practices was conducted using univariate models. The analysis found that *Campylobacter* positivity/high prevalence was associated with increased feed conversion, increased adjusted prime cost, and decreased paw quality/increased percent hock burnt. Conversely, the variables of increasing average weight, increasing average daily gain, increasing feed conversion adjustment factor values, practice of litter treatment with different chemicals, increasing house temperature, increasing air litter temperature, the condition of air ammonia level (being less than 25 ppm) and litter amendment being used at proper rate were all associated with *Campylobacter* negativity/low prevalence. These findings identified several variables that may significantly influence the prevalence of *Campylobacter* on U.S. commercial broiler farms. Evaluation of these specific variables, alone or in combination, under laboratory and/or commercial settings is warranted in order to definitively determine their impact and to develop and implement effective interventions for controlling *Campylobacter* on poultry farms.

The Fly hypothesis
for *Campylobacte*r transmission revisited

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Campylobacter epidemiology remains complex and drivers remain subject to debate. Fly transmission might explain *Campylobacter* epidemiology.

Hypothesis 1 Contamination occurs through fly transmission from faeces or carrion to food that is about to be eaten rather than earlier in the food chain. It explains the discrepancy between source attribution and case-control studies that use exposure data. Most disease is sporadic. Hypotheses 1 remains largely untested. The epidemiology methods examining for fly transmission are poorly developed.

Hypothesis 2 suggests that biosecurity in general and flies in particular play an important role in the contamination of chicken flocks from agricultural sources of animal faeces. The hypothesis has been tested by putting fly screens on well-run chicken houses and demonstrating reductions in *Campylobacter* colonization. It provides an explanation for why chicken meat is more contaminated in summer months (i.e. when flies are more active). Evidence from testing Hypothesis 2 provides some confidence that flies may be involved in directly contaminating food (Hypothesis 1).

Both hypotheses suggest that the seasonality of infection is strongly influenced by seasonal changes in fly populations and that the total burden of disease from this route is large.

Flies transmitted dysentery in the British Army in the early 20th Century. In Africa trachoma insecticide interventions to reduce fly transmission of chlamydia caused reductions in diarrhoea. Fly transmission is discussed in Environmental Health but not Microbiology and Microbial Epidemiology textbooks. Estimating fly exposure is difficult, source attribution has limited value and designing interventions complex. *Campylobacter* are isolated from flies in the environment, does not survive for long in fly feces or through maggot development but can be experimentally transmitted to chicks by flies. The fly hypotheses are attractive because they explain features of *Campylobacter* infection that are not easily explained by alternative hypotheses.

The *Campylobacter jejuni* contamination level in houseflies after exposure to contaminated faeces

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Houseflies have been found to carry *Campylobacter jejuni* and play a role in infection of poultry flocks. This study aimed to elucidate the quantitative Campylobacter level in naturally infected houseflies depending on their previous exposure to Campylobacter-contaminated faeces in terms of Campylobacter level and duration.

Houseflies (*Musca domestica*) reared under laboratory conditions were placed in 250-ml cups containing 5 g of chicken faeces spiked with 3, 4, 5 or 7 Log CFU *C. jejuni*. Sixteen houseflies were added to each cup at a time. After approx. 1 h of exposure to the faeces, four flies were removed from the cup for direct enumeration of campylobacter in each fly by plate spreading. Another four flies were transferred onto Abeyta-Hunt-Bark (AHB) agar plates (9 cm) to assess if the fly would contaminate the surface with Campylobacter. After 1 h on the AHB plate, each fly was tested for Campylobacter content and the AHB plates were incubated. This procedure was repeated after approx. 4 h of exposure for the remaining eight flies.

The percentage of houseflies positive for Campylobacter as well as the Log_{10} CFU recovered per fly depended on the contamination level with 90.0% (n=80), 48.4% (n=64), 6.3% (n=48) and 0% (n=16) of flies being Campylobacter-positive when exposed to 7, 5, 4, and 3 Log_{10} CFU with a mean (±SE) of 2.0±0.1, 0.8±0.1, 0.3±0.0 and 0 Log_{10} CFU recovered per Campylobacter-positive fly, respectively. Campylobacter seemed to be taken up readily as there was no significant effect of exposure time (1 vs. 4 h).

The surface of the AHB plates was only contaminated by houseflies previously exposed to either 5 or 7 Log_{10} CFU. The results support that houseflies are likely to become contaminated with Campylobacter if exposed to faeces containing >4 Log_{10} CFU.

Glucose utilisation by *Campylobacter jejuni* strains isolated from farm associated Norway rats

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Introduction: Until recently, *Campylobacter jejuni* has been considered unable to utilize glucose as a growth substrate. In this study, we document presence of genes of the Entner- Doudoroff (ED) pathway in 36 of 119 sequenced genomes of *C. jejuni* strains isolated from farm-associated Norway rats in 8 farms over 2 years. This is in contrast to a very low percent of ED positive *C. jejuni* strains identified in the PUBMLST/campylobacter database. Functionality of the ED pathway in these strains and ability to colonise chickens is addressed.

Material and methods: Utilisation of glucose was assessed by monitoring bacterial growth and glucose concentration over 72h. To monitor chicken colonisation, nineteen day-old Ross chickens (n=7/strain/ slaughter time) were infected with approximately 10^7 CFU by oral gavage. Caecal *Campylobacter* were quantitated by CFU at 3 and 7 days of post infection.

Results: Isolates included several diverse ED types; 63.8 % belonged to the closely related ED type 2/3. Utilisation of glucose was shown to support growth in DMEM minimal media and prolong survival in Mueller Hinton broth. Two ED positive strains tested were able to colonise chickens, although only to an intermediate level (mean ~ 10^5 CFU/g caecal material) compared to 5 ED negative strains that colonised to >10^7 CFU/g and 2 strains (ED negative, poor colonisers) for which only 1 chicken was colonised each.

Conclusions: These results show that farm-associated rats are frequently colonised by strains of *C. jejuni* that carry a functional ED pathway. The impact of the limited ability of these strains to colonise chicken caecum is being further assessed by analysis of the caecal microbiome and recovered strains.

Multilocus sequence typing of *Campylobacter jejuni* along poultry food chain in Poland

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C. jejuni is widely present among different warm-blooded animals; however, poultry and poultry products are the most important risk factors for acquiring the illness caused by these bacteria in humans. Most campylobacteriosis cases occur sporadically and many of infection sources remain unknown. To estimate the proportion of human infections attributed to different kinds of food, various typing methods have been applied to distinguish *Campylobacter* isolates.
In the present study, multilocus sequence typing (MLST) was used to determine the genetic diversity of *C. jejuni* from chicken caeca (n = 22), carcasses (n = 22), meat (n = 24), and humans (n = 26). Among 94 isolates, 47 different sequence types (ST) were found, including 5 new STs. The most prevalent sequence type ST464 was common among the isolates from caeca (n = 4) and carcasses (n = 4) but also detected in few strains of the remaining sources. ST137 was mainly associated with *C. jejuni* from meat (5 out of 24 strains) and it was not identified in other matrices. In human isolates, two predominant STs, i.e. ST51 (n = 3) and ST464 (n = 3) were detected. Seventy six STs were grouped into 17 clonal complexes (CC) and 5 of them (CC464, CC21, CC257, CC45, CC353) covered 60.5% of all isolates. Additionally, three most frequent clonal complexes CC464 (n = 13), CC21 (n = 11) and CC257 (n = 8) represented strains from all four origins.
In conclusion, *C. jejuni* population tested was diverse with a high number of STs identified in all sources. MLST analysis revealed that the majority of the isolates were assigned to globally distributed clonal complexes or had a strong link to the human variants CC21, CC45 and CC257.

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Quantitative analysis of *Campylobacter* spp. contamination in chicken slaughtering lines by "label tracking method" in eastern China

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Campylobacter contamination of poultry meat occurs vastly and inevitably in chicken slaughtering line. The aim of this study was to determine the quantification of *Campylobacter* spp. contamination levels in chicken slaughtering lines by a "label tracking method", in eastern China. From the six critical slaughtering steps in 4 slaughtering house, a total of 1 260 samples were collected in 236 sampling chicken. Results showed that *Campylobacter* may propagate in each slaughtering step with a high prevalence (83.05%). Both the highest *Campylobacter*-positive rate and the concentration of isolates were detected at the point of evisceration (97.46%; 2.80 ± 2.52 Log10 CFU/100 cm2), the *Campylobacter* contamination was mitigated after washing and chilling. However, after flash-freezing, the positive rate of *Campylobacter* was return to a high value while the concentration was reduced, and frozen storage has been confirmed allowing a mitigation on *Campylobacter* prevalence, qualitatively and quantitatively. Furthermore, the dynamic variation rule of *Campylobacter* prevalence obtained from different slaughtering environments was consistent with the rule identified from the corresponding slaughtered poultry. *Campylobacter* isolates obtained from different slaughtering processes in one slaughter-batch chicken were shown with above 90% homology, pointing to a potential source of contamination. Interventions are needed to minimize *Campylobacter* contamination, especially in washing, chilling, and frozen storage processes. Our study highlights the quantification of *Campylobacter* spp. contamination levels in broilers slaughtering line, which would provide quantitative data for the further studies on poultry meat safety control.

Investigations into the association of *Campylobacter* contamination in chicken carcase and caeca samples in the United Kingdom

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Campylobacter is the leading zoonotic enteric disease in Europe. The main source of infection is believed to be the handling, preparation and consumption of chicken meat. *Campylobacter*-colonised chickens entering the slaughterhouses are considered to be the main source of carcase contamination and it has been suggested that low numbers of *Campylobacter* in the caeca results in substantially reduced carcase contamination.

This study aimed to investigate the concentration of *Campylobacter* contamination in caeca and carcase samples from 732 slaughter batches sampled at 19 abattoirs over a three years period in the UK (2012-2015). One caeca and one carcase sample was collected per batch and tested to detect, quantify and speciate *Campylobacter* following methods described in ISO 10272: 2006.

The prevalence of *Campylobacter* on carcases (76.0%) was very similar to the prevalence obtained in caeca samples (75.3%) and results from both samples showed a good level of agreement (k=0.69). The level of *Campylobacter* contamination was much higher in caeca than in carcase samples and most (95.8%) of the highly contaminated carcases (>1,000 cfu/g) were obtained from batches with high loads of *Campylobacter* in the caeca (>10^6 cfu/g). Although the number of *Campylobacter* on the carcase samples was correlated with the concentration of contamination in the caeca, the strength of this association varied within and between abattoirs, suggesting differences in hygiene control.

The results from this study show that a reduction of the level of carcase contamination could be obtained by interventions aimed at reducing the concentration of *Campylobacter* in the colonised birds.

Identification of genetic determinants that facilitate *Campylobacter jejuni* survival during poultry processing and storage

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Campylobacter jejuni is a leading cause of bacterial-derived gastroenteritis with approximately 1.3 million human infections in the United States, annually. It is commonly found in the gastrointestinal tract of chickens and the primary route of human infection in developed countries is through consumption of undercooked poultry. Though *C. jejuni* is a microaerophile with optimum growth at 42°C, the microorganism is capable of surviving the atmosphere and temperatures encountered during poultry processing and storage. To identify determinants necessary for survival, we screened approximately 8,000 transposon mutants for those that were viable when grown microaerobically at 37°C, but became unculturable following 72 hours of cold (4°C), aerobic storage. Using this method, we identified 185 strains that were not cultivatable following storage. Following this primary screen, the magnitude of the survival defect was calculated for each of those mutants. One mutant that was confirmed to have a survival defect contained an insertion in *virB8* (Cjj81176_pVir001), which encodes a putative pore-forming secretion protein. Quantitation found that the *virB8* transposon mutant exhibited a significantly decreased survival rate of 0.45%, a 6-fold decrease compared to wild-type DRH212. Complementation with plasmid-encoded *virB8* restored survival to levels indistinguishable from wild-type. Using supernatant assays, we identified proteins of interest that were present in wild-type and complemented mutant supernatants, but were absent in *virB8* mutant supernatants. This data indicates that proteins secreted through the VirB8 pore may be involved in survival during cold, aerobic storage.

Quantitative surveys of *Salmonella* and *Campylobacter* on retail raw chicken in Yangzhou, China

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To assess the risks to consumers from *Salmonella* and *Campylobacter* in whole raw chickens sold in Yangzhou City, 12-month quantitative surveys were performed in succession. In this study, 480 samples were collected from supermarkets and wet markets from 2011 to 2013. The examination method of *Salmonella* was optimized from the most probable number (MPN), and the level of *Campylobacter* contamination was tested using the direct plating method. These results showed that the positive rates of *Salmonella* and *Campylobacter* were 33.8% and 51.3%, respectively, and the corresponding mean values of enumeration were 0.524 MPN/g and 1473.49 colony-forming units/g. For prevalence and loads of *Salmonella*, there was no significant difference between supermarkets and wet markets. However, for *Campylobacter*, the contamination level of wet markets was greater compared to supermarkets. Seasonality was observed in both qualitative and quantitative studies for both pathogens, with summer being the high-incidence season. Diversity among *Salmonella* isolates was high in terms of serovar, and the dominant serotypes were *Salmonella* Typhimurium (34.6%) and *Salmonella* Enteritidis (16.7%). Diversity of *Campylobacter* isolates demonstrated that *Campylobacter jejuni* (45.5%) and *Campylobacter coli* (30.9%) were the most common species, except for the mixed contamination. Survey results indicated that there was a need for more interventions to minimize the exposure of consumers to *Salmonella* and *Campylobacter*.

Campylobacter spp. in retail poultry and bovine meat in Italy

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The incidence and prevalence of campylobacteriosis have increased worldwide in the last years. Several studies have pointed out that the consumption of animal products is the main source of infection. The objectives of this study were to determine the prevalence of *Campylobacter* spp. in poultry and bovine retail-meat in Italy, and to assess drug resistance and genomic variations of the isolates. During the years 2015 and 2016, 1,243 poultry meat samples and 1,203 bovine meat samples were collected at the retail outlets. *Campylobacter* was detected using ISO procedures and susceptibility profiles were determined by microdilution method in accordance with the CLSI. The genomic assessment was carried out using PFGE and MLST. 17.38% of poultry and 0.58% bovine meat tested positive for *Campylobacter* revealed 135 *C. jejuni* (58.45%) and 88 *C. coli* (41.55%). The highest resistance in chicken strains was observed for Ciprofloxacin (84%), Nalidixic Acid (76.5%) and Tetracycline 65%. Multi-drug patterns were also observed. PFGE analysis showed 73 genotypes for *C. jejuni* and 54 for *C. coli*. Using 95% of similarity values, 8 clusters for *C. jejuni* and 4 clusters for *C. coli* were obtained. MLST analysis revealed that the most prevalent CCs for *C. jejuni* were 353, 354, 21, 206 and 403 with 20 different sequence types (STs); while the most prevalent MLST profile for *C. coli* was 832ST-828 complex. The most common flaA alleles were, respectively, 287 and 66 for *C. jejuni* and *C. coli*. Our study confirms that poultry meat is the main source of campylobacteriosis. On the other hand, the red meat had a very low level of contamination suggesting a minor role in the transmission. The high presence of *Campylobacter* in retail chicken meat, paired with the increased resistance to the antimicrobials with several multidrug resistance profiles is alarming, and represent a persistent threat to public human health.

Campylobacter spp. prevalence and levels of contamination in poultry meat and bovine meat preparations marketed in Italy

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Campylobacter is the most commonly reported zoonotic agent in European Union and poultry meat is considered to be the most important source of human campylobacteriosis.
Since other sources of human infections may be underestimated and data on *Campylobacter* contamination in poultry are mainly limited to slaughterhouse, the present survey intended to evaluate prevalence and levels of contamination of *Campylobacter* spp. in poultry meat and in bovine minced and knife-cut meat preparations at retail in Italy.
A sampling programme was designed considering three Italian macro-regions (Northern, Central and Southern Italy); a total of 1243 poultry meat and 1203 bovine meat preparations (689 hamburgers and 514 Italian traditional knife-cut meat preparations) were sampled.
Campylobacter detection and enumeration were carried out according to ISO 10272:2006. *Campylobacter* species were identified by multiplex PCR assay.
Prevalence was 0.58% in bovine meat preparations and 17.38% in poultry meat samples. Isolates were identified as *C. jejuni* (58.45%) and *C. coli* (41.55%). Levels of contamination were low in bovine meat preparations (1 sample - 0.08% above the limit of quantification of 10 CFU/g), while contamination in poultry meat were above the limit of quantification in 9.73% of samples.
Knowledge of prevalence and levels of contamination of *Campylobacter* spp. in food at the retail level is crucial for the evaluation of exposure of consumers. Data collected in the present survey are large enough to be representative of the entire Italian territory.
Results indicate that bovine meat preparations are rarely contaminated by *Campylobacter*; nevertheless Italian traditional knife-cut meat preparations are commonly eaten raw, therefore even small percentage of samples contaminated can suggest that public health risk remains. High prevalence and greater levels of contamination have been found in poultry meat at retail, confirming that this food may be the main source of human campylobacteriosis.

Quantitative Relative Risk Characterization of *Campylobacter* Contamination in Chicken Meat from Formal and Informal Urban Markets in Alexandria, Egypt

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Campylobacter is one of the top leading causes of diarrheal illnesses worldwide. Infection in humans is considered to occur mainly via foods of animal origin, especially consumption of contaminated chicken. The unhygienic practices at informal wet markets continue to pose significant source of contamination of fresh chicken meat. This study provides the first quantitative microbiological assessment of *Campylobacter* contamination levels in chicken meat sampled from informal wet markets (n=92) versus those from retail supermarkets (n=138) across the metropolitan city of Alexandria, northern Egypt.

Campylobacter was detected in 72.17% (166/230) of the chicken samples. *Campylobacter* was detected in 77.78% and 68.57% of chicken meats from informal wet-markets and retail supermarkets, respectively. *Campylobacter* contamination count was ≥ 1000 CFU/g in 35.5% (32/90) of chicken from informal wet-markets as compared to only 10% (14/140) in samples from retail supermarkets. The contamination levels with *Campylobacter* in chickens sold at informal markets were significantly higher than in retail supermarkets and this could be a driver behind higher foodborne exposure to such important zoonosis. In the future, more microbiological data combined with consumer practices in Egypt will be gathered in order to develop a quantitative risk assessment model.

Host origin predicts colonization ability in *Campylobacter jejuni*

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C. jejuni is a common component of the gut microbiota of chickens and a wide range of wild birds, for which previous studies have shown a strong host-association among sequence types (STs). This contrasts with the observation that in farm animals, several STs can co-exist in multiple hosts. Here, we compared colonization dynamics *in vivo* between three *C. jejuni* isolates from host-specific lineages (song thrush isolate from ST-1304; mallard isolate from ST-995 complex, chicken isolate from ST-21 complex) in a natural host, the mallard (*Anas platyrhynchos)*. We used real-time PCR (qPCR) and culturing of bacteria from various segments of the gut of infected animals to monitor infection. The genomes from strains from various host groups were sequenced and a comparative pan-genomic approach with an additional 134 *C. jejuni* whole genomes was used, using cloud-based computing (MRC CLIMB), to identify suitable qPCR targets to allow monitoring of infection dynamics for each strain *in vivo*. In 18 days infection experiments, the song thrush strain was not maintained stably and cleared after 10 days whereas both the mallard and chicken strains were observed to be able to colonize. However, when a chicken strain was allowed to colonise the birds for 5 days prior to co-infection with a mallard strain, it was found to be outcompeted. Interestingly, when a mallard strain was allowed to colonise the birds for 5 days prior to co-infection with the chicken strain, the two strains were able to co-exist at similar levels only 5 days after introduction of the chicken strain. This suggested mutualism of the two strains or different intra-host microniches. Our study is the first report infection dynamics of various lineages of *C. jejuni* in wild ducks and contributes to a better understanding of the ecology of host generalism and specialism in naturally-occuring foodborne pathogens.

Occurrence of zoonotic *Campylobacter* species in cattle and sheep farms

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In a previous study (2003-2006) carried out in ruminants in the Basque Country (Northern Spain), 62.1% of cattle herds and 55.0% of sheep flocks were *Campylobacter*-positive, identifying *C. jejuni* and *C. coli* in 21.8% and 5.9% of the farms, respectively. Then, all thermotolerant *Campylobacter* species were targeted and only one isolate per positive farm was identified, so that species like *C. hyointestinalis* in cattle or *C. lanienae* in sheep contributed to the high prevalence of the genus *Campylobacter*. Now, 10 years later (2014-2016), a similar number of farms (115 dairy sheep, 104 beef, 82 dairy cattle) were surveyed but targeting only the main zoonotic species and performing a more exhaustive analysis of isolates. Rectal faeces were cultured on a selective chromogenic medium (CASA, BioMerieux) at 42ºC, and a loopful of bacterial culture was screened for the presence of *C. jejuni* and *C. coli* in a multiplex Real-Time PCR. Individual isolates were then selected from positive samples for MLST characterization. Presence of any of the two species was detected in 82.3% of cattle herds and 54.8% of sheep flocks. The most frequent species was *C. jejuni*, present in 81.2% of cattle herds and 45.2% of sheep flocks, whereas *C. coli* was found in 9.7% of cattle and 17.4% of sheep farms. Both species were present in 8.3% of the tested farms. Preliminary MLST results showed that isolates were genotypically diverse. The apparent increase in prevalence estimates compared to the previous study can most probably be ascribed to changes in methodology rather than reflect a real increment. Absence of standardised approaches to sampling and detection methodologies hampers direct comparison of data among studies. Still, results confirmed that cattle and sheep can pose a risk for human infection.

Campylobacter jejuni at the level of a coastal catchment in France

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Campylobacter jejuni is the most common causative agent of human bacterial gastroenteritis and is frequently disseminated through surface water. The aim of this study was to evaluate its occurrence and diversity in a French shellfish-harvesting area and the upstream livestock farming-intensive catchment.

Methods: From February 2013 to January 2015, shellfish (n=120), seawater (n=12) and sediment (n=14) samples were collected in La Fresnaye Bay, Brittany, in addition to water samples (n=96) collected at the outlet of the four main sub-catchments.
The detection of Campylobacter sp. was investigated using qPCR, the EN.ISO.10272 culture method and a selective passive-filtration approach and *Campylobacter* species were identified by MaldiTof. Among the *C. jejuni* collection (n=298), 122 isolates were analyzed by Comparative Genomic Fingerprinting (CGF40) and 39 by MultiLocus Sequence Typing (MLST).

Results: A weak prevalence of *C. jejuni* was observed in the shellfish samples (0.8%) and in the seawater and superficial sediment samples contrary to a high prevalence at the outlet of sub-catchments (from 45.8% to 66.7%).
A high genetic diversity of *C. jejuni,* with 45 different CGF40-90% (based on >90% CGF40 fingerprint similarity) profiles was observed within the isolates. 28%, 15% and 57% of them have profiles commonly found in poultry and bovine reservoirs, rarely found or absent in these reservoirs, respectively. Furthermore, only 23.8% of isolates have profiles found in humans. The MLST data support that another source such as wild birds could be the main contamination source (e.g isolation of ST-177, 583, 677, 1286). Furthermore, *C. jejuni* was isolated in gull and goose feces on this site.

Conclusion: *C. jejuni* has commonly been found in rivers but rarely in the downstream shellfish-harvesting area. The sources are likely derived from agriculture and wildlife. Next step will be the design of potential source attribution markers targeting wild birds for an application to environmental samples.

Prevalence and antimicrobial resistance of *Campylobacter* spp. found in gulls, *Laridae*, wintering in Zagreb, Croatia

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A total of 173 gulls of 4 species (Yellow-legged Gull, *Larus michahellis*; Black-headed Gull, *L. ridibundus*; Caspian Gull, *L. cachinanns*, and Common Gull, *L. canus*) were captured using cannon net, in a period between November 2016 and March 2017 on Zagreb city garbage dump (45.45 N 16.01 E) in order to collect cloacal swabs. Taken swab samples were tested for the presence of thermophillic *Campylobacter* spp. using EN ISO 10272-1 method while the species determination was performed by multiplex PCR. Highest prevalence was found in Common Gulls (33,33 %) followed by Black-headed Gulls (22,89 %), Caspian Gulls (20,00 %) and Yellow-legged Gulls (18,37 %). Among 39 positive samples the most prevalent was *C. jejuni* (31 or 79,5 %) followed by *C. lari* (7 or 18,0 %) and *C. coli* (1 or 2,5 %). To our knowledge this is the first data on occurrence of *Campylobacter lari* in Croatia.

Antimicrobial susceptibility testing was determined for six antimicrobials (erythromycin, ciprofloxacin, tetracycline, gentamicin, nalidixic acid and streptomycin) by microdilution method (SensititreTM EUCAMP2, Thermo scientific) on 15 *C. jejuni*, 7 *C. lari* and 1 *C. coli* isolated strains. EUCAST ECOFFs values and EURL-AR interpretative criteria were used for *C. jejuni* and *C. coli*, while for *C. lari* same criteria as for *C. coli* were used. All of the tested isolates were resistant to ciprofloxacin, streptomycin and nalidixic acid. All of the *C. jejuni* and *C. coli* isolates were resistant to gentamicin, while 53,3 % of *C. jejuni* and 28,6 % of *C. lari* were resistant to erytromycin. Furthermore, 22,2 % of *C. jejuni* were resistant to tetracycline. The observed multi-resistance of the thermophillic *Campylobacter* contributes thesis of the importance of gull species as an important reservoir of *Campylobacter* and as a source of possible infection for animals and humans.

Genetic diversity of *Campylobacter coli* and *Campylobacter lanienae* from wild boars from a metropolitan area in northeastern Spain

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The increasing abundance of wild boar (*Sus scrofa*) and its adaptation to urban and peri-urban areas is a worldwide phenomenon, resulting in an increased risk of disease transmission between wildlife, domestic animals and humans. Campylobacteriosis is the most commonly reported food-borne disease in the EU. In our previous studies, up to 60% of the wild boars sampled at the metropolitan area of Barcelona (NE Spain) carried thermophilic *Campylobacter* spp. Here, we determined the genetic diversity of these *Campylobacter* spp. isolates by flaA-RFLP, ERIC-PCR and PFGE. *Campylobacter* isolates were obtained from faeces of 133 wild boars, either captured or hunted in three different areas of the Barcelona metropolitan area (one urban, one peri-urban and one rural). Overall, 70 *C. coli* and 188 *C. lanienae* isolates were analyzed. *C. coli* isolates were first screened for strain diversity by flaA-RFLP, whilst ERIC-PCR was used for *C. lanienae* isolates since they were not typable by flaA-RFLP. A selection of isolates from the different profiles obtained with either method were further typed by PFGE using *Sma*I and *Kpn*I enzymes. A 71% and a 9% of *C. coli* and *C. lanienae* isolates were typable with both enzymes, respectively. A 57% of all tested isolates were typable with *Sma*I and overall, this enzyme was more suitable than *Kpn*I for typing *Campylobacter* species from wild boar origin. A high genetic diversity was observed amongst both *Campylobacter* species. However, some isolates with the same or highly similar pulsotypes were found among the three sampling areas, suggesting that same *C. coli* and *C. lanienae* clones are circulating in wild boars from urban and rural areas. Close contact between wild boars and humans, which occurs in the studied areas, increase the risk of transmission of zoonotic agents, such as *Campylobacter*.

Antimicrobial susceptibility and putative virulence factors among campylobacteria isolated from animals in Valparaiso, Chile

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Campylobacter jejuni and *Campylobacter coli* have been worldwide recognized as the species most commonly causing food-related diarrhea. However, other *Campylobacter* species together with *Arcobacter* spp. and *Helicobacter* spp. are also recognized as emerging pathogens although they are not frequently isolated by traditional culturing methods and have been very little studied. In this sense, it is necesary the use molecular detection and identification methods in parallel to culturing methods.

It has been suggested that pet ownership significantly increases the risk for human *Campylobacter* infection, despite common sources of infection and directionality of transmission between pets and humans are still unknown. Moreover, *Campylobacter* spp. have become increasingly resistant to antibiotics probably due to antibiotic usage in both animal agriculture and human medicine.

This study assessed the prevalence of campylobacteria in faeces of dogs (n=61), cats (n=7), birds (n=33, mainly raptors), and wild animals (n=2). In 21 of them (20.4%) campylobacteria were detected by PCR, mainly dogs (14.6%), while only in six (5.8%) campylobacteria were isolated by culturing, i.e. *Campylobacter upsaliensis* (n=4, dogs), *C. jejuni* (n=1, dog) and 1 *Arcobacter butzleri* (n=1, chiken). None of the isolates was resitant to ciprofloxacine, but two of them were resitant to eritromycin, i.e. *A. butzleri* (MIC=8) and a *C. upsaliensis* (MIC=128). Regarding the virulence factors, only the *C. jejuni* isolate was positive for *cdtC* and *cadF* genes and the *A. butzleri* isolate, for *cadF* and *ciaB* genes.

This is the first study to assess the presence of campylobacteria in animals from Valparaiso, Chile, confirming the risk of transmission of these zoonotic bacteria from pets to ownwers, as well as the presence of virulence factors and resistance to antibiotics used for treatment in humans among them. These results warrant further studies to assess their relationship with human isolates as well as to confirm their importance as pathogens.

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Characterization of *C. jejuni* isolates from western jackdaws

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Wild birds have been frequently shown to carry *Campylobacter* and are thought to act as wildlife reservoirs also contaminating food production farms. However, relatively little is known about the types found in different bird species and environments. Western jackdaw has become one of the most common bird species in some urban and agricultural surroundings in Finland and thus of concern also in a public health perspective. In this study we isolated *C. jejuni* from western jackdaws (*Corvus monedula*) from two small cities, one from Southern and one from Western Finland during a 5-month period (September-February) in 2014-2015. 212 fecal droppings were collected from which 91 (43%) were found to be positive for *C. jejuni* after direct culture on mCCDA. MLST profiles were successfully completed for 88 *C. jejuni* isolates representing 62 different sequence types (STs), from which 46 were novel in the PubMLST database. Thus, great diversity was found among the STs and the majority of the isolates (n=55) represented new sequence types. ST-1282 and ST-6460 were most frequently identified and most widely distributed both temporally and geographically. Previously ST-1282 has been reported in the PubMLST database to originate from a wild bird in Sweden and environmental waters in Luxembourg. ST-6460 has been reported from a gastroenteritis patient in Sweden. Other types found among *C. jejuni* from western jackdaw and previously reported from human stool samples (according to the PubMLST database) included ST-1539, ST-6589 and ST-6590. However, overall STs previously reported in human disease accounted for only 9 out of the 88 isolates (10%). Furthermore, none of these STs have been detected from Finnish chicken farms although sampling was partly targeted close to chicken farms. We conclude that western jackdaw is a possible yet unlikely source of campylobacteriosis in humans. More detailed genome-wide characterization of the isolates will be presented.

Poster session

« Control strategies for *Campylobacter sp.* »

Campylobacter colonization in broilers raised under different management concepts

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Since 2015, a joined effort of Dutch retailers and the poultry industry led to an almost complete switch for the retail market from conventionally reared fast-growing broilers to slower growing birds in alternative systems, with differences *e.g*. in broiler type, stocking density and dark period.
Although this transition has a positive impact on animal welfare and fewer antibiotics are used in slower growing birds, it also raised concerns regarding the risk of *Campylobacter*. Age is a risk factor for *Campylobacter* colonization in poultry. Because slower growing birds are being slaughtered at higher ages (depending on the market concept between 45-56 days) compared to traditionally reared broilers (37-43 days), it was expected that slower growers are more frequently *Campylobacter* positive at slaughter and may lead to increased *Campylobacter* contaminated poultry meat.
To assess the percentage of *Campylobacter* positive flocks, broilers were sampled at two locations of a Dutch slaughter plant. Over a period of 26 weeks, between May-November 2016, flocks from both concepts were tested for *Campylobacter*. Of every flock, a pooled sample of 30 caeca was examined by direct plating on CCDA plates that were micro-aerobically cultured at 41,5°C for 48 hours. Of 378 flocks that were screened, 150 were slower growing flocks of which 72% (confidence interval 64-79%) were *Campylobacter* positive. Of the fast growing flocks 79% (confidence interval 73-84%) were positive. Although this difference was not statistically significant, it confirmed previous findings as well as private data from the slaughterhouse.
In conclusion, although the slower growing chickens are slaughtered at an older age, the percentage of *Campylobacter* positive flocks was not higher compared to conventionally fast growing chickens. Possible factors that may account for this are differences in breed and/or farm management. Further exploration may be valuable in providing supporting tools for the control of *Campylobacter*.

Efficient prevention of *Campylobacter* spp. entrance in broiler houses improves flock performance at slaughter

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Campylobacter spp. is recognised as a commensal in chickens and there is no recorded effect on bird weight gain or feed conversion at farm level. A total of 14,664 commercial broiler houses were sampled for the presence/absence of *Campylobacter* spp. Samples were collected from five different management areas across the UK from houses supplying a large poultry integrator. All houses were sampled prior to partial depopulation at approximately 31 days of age using a boot swab and *Campylobacter* presence was detected by qPCR. Results from management area 1 (*n*=5350) were aligned to bird performance indicators and statistical analysis conducted using analysis of variance (ANOVA). Significant differences in mean body weight at day 0, 7, 14, 21, 28 and 35 days (after correction) were observed between houses testing positive or negative for *Campylobacter* spp. From day 14 through to slaughter flocks from *Campylobacter* negative houses achieved significantly greater weights for age performance than *Campylobacter* positive houses. Average daily gain (g/day) was also greater. This study emphasizes that applying stringent and efficient biosecurity measures to reduce *Campylobacter* spp. entrance to broiler houses could improve the economic outcome of broiler production. To our knowledge this is the largest, commercially applicable dataset collected at farm level to have shown a negative association with *Campylobacter* spp. and commercial broiler flock performance. Further study is required to understand the link between *Campylobacter* spp. and flock performance.

Optical flow predicts *Campylobacter* colonisation of broiler flocks at 7-10 days of age.

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Introduction: The influence of *Campylobacter* colonisation on the overall gut health, welfare and behaviour of broiler flocks was investigated using optical flow, alongside other more traditional measures of welfare and performance. Optical flow provides a continual automated means of assessing the movement of broiler flocks as a whole, by examining changes in the pixilation of camera images over time. Rather than relying on post-mortem assessment of the welfare of individual birds, the method allows for interventions in the management of living flocks.

Materials and methods: To date, 31 commercial flocks from the UK, representing 2 companies and 3 sites, have been monitored by optical flow until 30 days of age, with statistics calculated every 15 minutes. A combination of faecal samples and boot socks were used to test the flocks for *Campylobacter* colonisation at 21, 28 and 35 days of age using direct culture methods. The study is currently being extended to include more UK and European flocks.

Results: *Campylobacter* positive flocks from both companies investigated to date demonstrated lower mean optical flow (less average movement) and higher kurtosis (less uniform movement) measures, compared to *Campylobacter* negative flocks. These differences were detectable within 10 days of age and were independent of environmental temperature. In addition, whilst measures of pododermatitis and percentage mortality were not indicative, a small but persistent difference in weight was apparent between *Campylobacter* positive and negative flocks.

Conclusions: It is not yet clear whether *Campylobacter* directly causes the changes in flock behaviour detected by optical flow, or whether certain flocks are predisposed to becoming colonised by *Campylobacter*. Early prediction of *Campylobacter* colonisation however, draws attention to the management of young chicks within the first week of life, and allows for targeted intervention and management of living flocks.

Vaccination with nanocapsules derived from the ureA protein can induce protective immune responses in mice.

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Introduction: The urease enzyme is a major virulence factor for *H. pylori*, and therefore a primary target for vaccination. The enzyme consists of two subunits, A and B. We have expressed ureA as a recombinant protein in *E. coli*, and used the purified protein to construct protein- only nanocapsules. We then compared the vaccine efficacy of the nanocapsules with a well- studied vaccine, SL3261pyz97 (Gomez-Duarte *et al.,* Vaccine ,1998.16: 460-471).

Materials and Methods: The ureA gene was codon optimised and inserted into an *E. coli* expression vector. The purified protein was infiltrated into various sized mesoporous silica templates, the protein was cross- linked and the silica dissolved, leaving the protein- only capsules. Capsules of 50 and 500 nm were constructed. Nanocapsules of approximately 500 nm diameter were formulated with Titremax and used to vaccinate mice. As a positive control, recombinant *S. typhimurium* expressing ureA and ureB was also used. Mice were challenged with mouse-adapted *H. pylori* strain SSI. Bacterial loads and immune parameters were assessed.

Results: Vaccination with nanocapsules and adjuvant, and subsequent infection, resulted in a significant increase in stomach CD4 and CD8 cells over both the infection and adjuvant control. These increases were not evident in the mesenteric lymph nodes. Bacterial loads were assessed using real-time PCR of *H. pylori* 16S rRNA. Colonisation correlated with the cellular responses, with the nanocapsule plus adjuvant group giving a significant reduction in load compared to the control groups. This indicates that prior vaccination expands protective cell populations.

Conclusion: Nanocapsules of various sizes can be constructed from recombinant ureA protein. When used in conjunction with adjuvant, these nanocapsules can significantly enhance cellular responses, and this correlates with a reduction in Helicobacter colonisation. Future work will be aimed at maximising the efficacy of different sized particles.

Development of enterobactin antibody-based immune intervention strategies

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Siderophore-mediated iron acquisition has been targeted for iron-dependent pathogen control given its critical role in bacterial pathogenesis. Enterobactin (**Ent**), a representative catecholate siderophore that has the highest affinity for ferric iron, is an archetype for iron acquisition in Gram-negative bacteria. Our recent studies indicate that *Campylobacter* is an ideal model organism to explore innovative strategy of iron-dependent control. Lipocalins, the soluble host acute phase proteins, have been demonstrated to function as an innate bacteriostatic agent to control Gram-negative infections by interfering with Ent-mediated iron acquisition through their potent Ent-binding ability. This evidence has prompted us to hypothesize that Ent specific antibodies function as an effective bacteriostatic agent against infections caused by *Campylobacter* as well as other Gram-negative pathogens. To test this, in this study, we have optimized conditions for conjugation of Ent to two commonly used carrier proteins, keyhole limpet hemocyanin (**KLH**) and bovine serum albumin (**BSA**). The KLH-Ent conjugate was used to immunize rabbits, successfully generating Ent specific antibodies. In addition, we also immunized layers with the Ent-KLH conjugate, which triggered strong immune response and led to the production of Ent specific egg yolk antibodies. Together, we have developed an efficient method to make Ent conjugates and successfully generated different types of Ent specific antibodies, which provides a solid platform for us to develop and evaluate new vaccine and therapeutics against *C. jejuni* and other Gram-negative pathogens.

A proof of concept study demonstrates the reduction of *Campylobacter* levels using bacteriophages

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Campylobacter bacteriophages have the potential to reduce *Campylobacter* in the caeca of broiler chickens contributing to reduction in numbers. A recent "proof of concept study" was carried out in Australian farm and processing environments during commercial production. To demonstrate the proof of concept, farms were selected with the following criteria: (a) the absence of native phages (b) high *Campylobacter* counts (c) sensitivity to the selected phages to be trialled as assessed one week prior final pick-up. On Farm R a combination of four phages administered in tap water showed a significant reduction in *Campylobacter* levels that exhibited a range between log10 5.18 – 6.25 CFU/g for treatment compared to the control range of log10 6.05 – 8.15 CFU/g (P=0.02). However, the difference between treated and control birds was not statistically significant upon transport to the plant (caeca), but the majority of the Campylobacter levels in the treatment chickens remained lower than controls (range log10 5.14 – 6.62 CFU/g). On Farm D (the second farm) two selected phages showed good coverage and the farm was phage negative during pre-screening. However, at trial Farm D failed to meet the trial conditions due to the incursion of natural phage prior to treatment. Irrespective of this situation, the *Campylobacter* isolates (farm and the plant) recovered from the native phage infected shed from Farm D remained sensitive to the administered phages. Phages were not recovered from the carcasses of chickens of either farm.

Application of Bacteriophage to Control Contamination of *Campylobacter* in Chicken Liver

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Bacteriophages have gained recognition as therapeutic agents to control pathogens in livestock and poultry and represent a potential approach to control campylobacters in livers. In this study, virulent bacteriophages were applied to assess their potential to reduce contamination of *Campylobacter* in vivo and on retail chicken liver. Experimental infection of broiler chickens with liver origin C. jejuni (CLB104) resulted in efficient intestinal colonisation. By enrichment, the bacteria were also recovered from the livers (3 of 7 chickens) and other extra-intestinal organs (1 to 3 of 7 chickens). The application of phages ϕ3 and ϕ15 (8 log10 PFU/g) to liver homogenates containing either low (3 log10 CFU/g) or high (5 log10 CFU/g) *C. jejuni* inoculum at 4 °C for 48 hours resulted in modest but significant reductions in the viable counts ranging from 0.2 to 0.7 log10 CFU/g. Oral administration of phage C3 to chickens infected with *C. jejuni* CLB104 reduced *Campylobacter* counts in the caeca and ileum of 1.0 log10 CFU/g and 1.3 log10 CFU/g, respectively. However, since campylobacters colonisation in the ovals of chicken could only be detected by enrichment, assessment on the efficacy of phage therapy would require frequent and consistent colonisation of the bacteria in the extra-intestinal organs. Low-passaged *C. jejuni* CLB104 will be tested on its ability to re-colonize chicken's systemic organ and, presuming that they show better colonization, will be used in the subsequent phage treatment trial.

Bacteriophage therapy to reduce *Campylobacter jejuni* colonization in broiler chickens

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Since 2005, campylobacteriosis is the most commonly reported gastrointestinal disease in humans in the European Union and *Campylobacter* colonization in broilers is widespread and difficult to prevent. Bacteriophages prey and kill bacteria, are naturally occurring agents, ubiquitous in nature and their application in phage therapy could contribute to treat antibiotic resistant bacteria and can represent an alternative option to control *Campylobacter* in poultry. The aim of this work was the evaluation of the efficacy of phage therapy in reducing the contamination levels of *Campylobacter* in poultry. Thirty-six lytic bacteriophages were isolated and screened against a panel of 8 *Campylobacter* field strains and one NCTC-12662 strain. Two phages (Fi7 and Fi16), showing a broad host range, were chosen for the *in vivo* experiment. In particular, 75 broilers were divided into three groups (A, B and C) and phages were administered to animals of groups A and B in antiacid suspension at day 38 (Fi16) and 39 (Fi7). Phage concentrations were 10^7 pfu/ml (group A) and 10^8 pfu/ml (group B). Control birds (group C) received the placebo (antiacid solution) only. All broilers were killed at day 40 and *Campylobacter jejuni* was enumerated in cecal contents. *Campylobacter* counts from phage treated poultry were between 1 (group A) and 2 $\log_{10}$ cfu/g (group B) lower than the control group. Our findings provide evidence about the ability of phage therapy to control *Campylobacter* contamination in poultry and could contribute to significantly reduce the risk for humans to get infected with consumption of chicken meals. Moreover, phage treatment showed no visible adverse effects in chickens. More research is needed to improve the efficiency of phage therapy, to explore the robustness of the method when applied to modern chicken management systems and to assess the phage efficacy versus different *Campylobacter* field populations.

Identification of genetic determinants that facilitate *Campylobacter jejuni* survival during poultry processing and storage

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Campylobacter jejuni is a leading cause of bacterial-derived gastroenteritis with approximately 1.3 million human infections in the United States, annually. It is commonly found in the gastrointestinal tract of chickens and the primary route of human infection in developed countries is through consumption of undercooked poultry. Though *C. jejuni* is a microaerophile with optimum growth at 42°C, the microorganism is capable of surviving the temperatures and steps associated with poultry processing and storage. To identify determinants necessary for survival, we screened approximately 8,000 transposon mutants for those that exhibited decreased viability following 72 hours of cold (4°C), aerobic storage. From this screen, we identified 185 strains that were not cultivatable following storage, including an independent insertion in flgE2 (Cjj0081). Quantitation of this survival defect found that a flgE2 deletion mutant exhibits survival rates of 0.91%, a 2-fold decrease compared to wild-type DRH212 (1.7%). Similar to earlier studies, we found that the flgE2 mutant exhibits wild-type motility, indicating that the survival defect is not due to swimming-dependent aerotolerance. Since previous studies have shown that intestinal colonization by one strain of *C. jejuni* can prevent colonization by other strains, we hypothesized that colonization of a chicken with the flgE2 mutant may prevent colonization by survival-proficient wild-type *C. jejuni*. The flgE2 mutant was used to inoculate day-of-hatch chicks and one day later, the same chicks were inoculated with wild-type *C. jejuni*. After six days, chickens were harvested and *Campylobacter* were enumerated from the ceca. This will offer some insight into whether flgE2 *Campylobacter* mutants will be able to competitively inhibit wild-type *C. jejuni* growth, thus providing a possible method to decrease the occurrence of campylobacteriosis.

Effect of a combination of two feed additives against *Campylobacter* infection in commercial turkey farms

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Risk assessment studies pointed out that reducing the number of *Campylobacter* at the poultry primary production will have a great impact in decreasing the number of human campylobacteriosis. Therefore, implementation of control strategies at the farm is needed. This work aimed to evaluate the effect of a combination of a probiotic and an organic acid mixture added to feed on *Campylobacte*r contamination in French commercial turkey farms as few data are available regarding this production.

Two turkey farms with two buildings and naturally contaminated with *Campylobacter spp.* were studied. Animals in one building were fed with a control diet (additive free) and the ones in the other building were fed with the probiotic and organic acid mixture, during the whole rearing period. Ceca sampling was performed at slaughter at 12 (females) and at 18 (males) weeks of age. *Campylobacter* enumeration was performed by molecular analysis of caecal contents after DNA extraction and real-time qPCR. The comparison between control and treated group was performed using statistical analysis based on multiple comparison tests.

This field trial gave interesting results regarding the *Campylobacter* contamination of French commercial turkey farms. In the control groups, the level of contamination varied according to the farm, especially after 12 weeks of rearing (slaughter age of the females), but contamination of the males seemed less variable. Unfortunately, the combination of feed additives did not have the intended effect upon *Campylobacte*r contamination. Indeed, no effect on level of contamination was observed in the treated groups compared to the control groups. This nutritional strategy is therefore not efficient to reduce *Campylobacter* in commercial turkey farms.

Combination of water and feed additives against *Campylobacter* in free-range broilers

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Poultry is generally recognized as the most-important source for human campylobacteriosis. Implementation of control measures to reduce Campylobacter loads in broiler's intestines at the primary production is a way to reduce the incidence of this zoonosis.
No real effective solution has been found to date and feed or water treatments are still extensively studied in conventional broilers. However, there is a growing interest of consumers for free-range poultry which accounts for an increasing part of the consumption of broilers. The aim of this work was to test a combination of two commercial products, a clay-based product in feed associated with an organic acids mixture treatment of drinking water against natural Campylobacter colonization of free-range broilers during the whole rearing period.
Broilers reared in a French experimental farm were treated in feed and water during the last week of rearing (from day 71 to 78) and were compared to a control group. Campylobacter loads were assessed in caecal contents and on carcasses of broilers at slaughter following the decimal dilution method. The comparison between control and treated animals was performed using statistical analysis based on mean and multiple comparison tests
The flock was naturally contaminated at the end of the indoor rearing period between day 35 and day 42. The combination of products significantly reduced the contamination of 0.82 ± 0.25 Log10 CFU/g (p= 0.02) compared to the control group at the end of rearing at day 78. At slaughter, a slight but significant (p=0.01) reduction of 0.68 ± 0.24 Log10 CFU/g was obtained on the reduction of Campylobacter on carcasses compared to the control group These results need to be confirmed during several other field trials to ensure if it could be applied as a reliable control measure.

Antimicrobial activity of a novel commercial antimicrobial (Auranta 3001), against T6SS positive *Campylobacter jejuni* and *Campylobacter coli*

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Human campylobacteriosis is considered one of the most common foodborne diseases internationally. Poultry have been identified as the main source of infection accounting for 50-80% of human cases. Recently, highly virulent *Campylobacter* spp. positive for the Type VI secretion system (T6SS), which have increased abilities to adhere and invade the host gastrointestinal epithelium, have been reported. Therefore, new antimicrobials to supplement animal feed that able to control *Camplylobacter* species are highly sought after. To elucidate the effect of the Auranta antimicrobial *C. jejuni* strains and *C. coli* human and poultry isolates positive and negative for the *hcp* gene (presence indicative of a functional T6SS) were used. Disc diffusion assay was employed to assess the antimicrobial activity at different concentrations (2, 4, 8 and 10%). The microdilution broth method was used to determine the minimum inhibitory concentration of the antimicrobial. Minimum bactericidal concentrations were also determined. The effect of the commercial antimicrobial on the invasiveness of the *C. jejuni* and *C. coli* using the gentamicin protection assay was also investigated. Strong antimicrobial activities against both *C. jejuni* and *C. coli* were observed. Minimum inhibitory concentrations ranging from 0.25% to 1% and minimum bactericidal concentrations from 1 % to 2% were observed for *C. jejuni* isolates. Whereas, minimum inhibitory concentrations ranging from 0.125% to 2% and minimum bactericidal concentrations from 0.25 % to 2% were observed for *C. coli* isolates. HCT-8 cell invasion assays also showed that the commercial antimicrobial modified the invasion capabilities of the strains. The present study characterised the effect of a novel feed antimicrobial from Auranta. The antimicrobial activity against both human and poultry isolates of *C. jejuni* and *C. coli* show its usefulness as a feed additive alternative to antibiotics for the prevention/treatment of *Camplylobacter* infection.

The mechanism of anti-*Campylobacter* activity of phytochemical formulations from alpine and karst plants, traditionally used against intestinal disorders

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With a high prevalence of resistant *Campylobacter jejuni* and its multidrug resistance still rising, the investigation of novel anti-*Campylobacter* agents is much needed. Recently, new control strategies are increasingly focusing on natural antimicrobials from plants as safe for consumers. We focused on ethanol and/or hexane extracts and their fractions from the alpine and karst plants, traditionally used against intestinal disorders, as are *Peucedanum ostruthium, Leontopodium nivale* ssp. *alpinum,* and *Satureja montana*. The mechanism of antimicrobial activity of these phytochemicals on *Campylobacter jejuni* is poorly understood, thus we aim to elucidate it by investigating their influence on key efflux pumps (CmeABC, CmeDEF and CmeGH) and potential resistance to natural alternative antimicrobials. After plant material preparation, using an accelerated solvent extraction method, with ethanol, 60% ethanol, dichloromethane and hexane, we analyzed their chemical composition by TLC, GC-MS and HPLC-MS/MS and determined the minimal inhibitory concentrations (MIC) of plant formulations with broth microdilution after dying with resazurin. Further we investigated their influence on: (i) efflux pumps with the ethidium bromide accumulation assay, (ii) bacterial membrane integrity using LIVE/DEAD BacLight assay; and (iii) sensitivity of mutants lacking activity in CmeABC, CmeDEF and CmeGH efflux pumps. The results exposed the influence of all tested extracts on the membrane integrity and/or their efflux pumps. MIC varied from 125 mg/L to more than 2000 mg/L depending on the extract. The most promising ethanol extract of *Satureja montana* showed good antimicrobial activity by influencing bacterial efflux pumps. A mutant lacking a functional efflux pump CmeGH was the most sensitive to this particular extract, whereas the efflux pump CmeABC had a bigger role in the resistance to other formulations. Studies of the mechanism of bioactivity are going on as they are essential for efficient application of identified anti-*Campylobacter* compounds.

The Effects of Thai-herb on *Campylobacter* colonization

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Campylobacter spp. is one of the leading causes of diarrheal disease worldwide. Poultry is the main reservoir of *Campylobacter* that can introduce the pathogen into human's food chain. Prevention and treatment of *Campylobacter* in poultry farm can be done by antibiotic treatment. However, increasing concern on antibiotic resistant microorganisms has prompt many countries to limit the use of antibiotics. Prophylactic use of antibiotic in food animals is banned in the EU countries since 2006. Many herbs have been shown to have antimicrobial activity, hence may be used as antibiotic alternative in controlling campylobacter in poultry. The purpose of this study was to determine the effect of a tradition Thai herb, pak taew (*Cratoxylum formosum*) influence on *Campylobacter* colonization. Methods of this study used Minimum Inhibition Concentration (MIC) test, Ferric Reducing Antioxidant Power (FRAP) assay, *In vitro* digestion model and Mitochondrial Toxicity (MTT) assay. MIC test was assay to determine the antibacterial activity. FRAP assay was used to evaluate the antioxidant activity. *In vitro* digestion model was investigated to determine the survival of *Campylobacter* under gastro-intestinal conditions. MTT assay was performed the toxicity of *Campylobacter* spp. with and without Thai-herbs on CaCO2 cell-line. In this study, *C. formosum* were ranged in concentrations of 0.30, 30 and 300 mg/ml. Results showed that Thai-herbs possessed strongly antioxidant activity and antibacterial activity. Survivals of *Campylobacter* were decreasing on treatment with *C. formosum* under gastro-intestinal tract and Caco-2 cell-line condition. *Campylobacter* spp. alone showed to have cytotoxicity to Caco-2 cell-line. However, *Campylobacter* spp. with *C. formosum* was decreasing on cytotoxicity to Caco-2 cell-line. Results from this study indicate that Thai herb illustrates the potential activity to reduce the survival of *Campylobacter* spp. under gastro-intestinal and decreasing the cytotoxicity from *Campylobacter* colonization to Caco-2 cell-line. In application, herbs extracts can be used as in animal feeds.

Efficacy of formulated carvacrol on *Campylobacter jejuni in vitro* and electronic microscopy approaches

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Campylobacter is well known as the leading cause of foodborne diarrheal disease worldwide, with *Campylobacter jejuni* and *Campylobacter coli* representing the most frequently involved species. The main source of infection is the meat from poultry origin mostly contaminated during evisceration. Thus, reducing *Campylobacter* concentrations in the intestinal tract and particularly in the caeca may help decreasing flesh colonization, and in this way reducing human infections by the bacteria.

Some natural substances have interesting antimicrobial properties. Studies have for example reported the antibacterial effect of carvacrol against *Campylobacter*.

As essential oils compounds are often absorbed before they reach the last part of the intestinal tract, they do not get to the site of *Campylobacter* growth. A new galenic formulation (Phodé Sciences, France) has been created to resolve this issue. This product contains a liquid formulated core based on carvacrol, and a specific solid carrier.

In the present study, we compared the efficacy of carvacrol and the formulated carvacrol, against *Campylobacter jejuni* ATCC 33291 using a broth microdilution method. The new formulation of carvacrol has the same efficacy as carvacrol alone ($P>0,05$).

We also compared the mechanism of action of both products by Scanning and Transmission Electron Microscopy. The new galenic formulation still showed the same results as pure carvacrol. Treated cells showed wrinkles, clefts and blisters. We also noticed, large membrane blebs caused by separation of the plasma membrane from outer membrane, with leakage of the cytoplasmic content into the intermembrane space.

We then undertook a follow-up of carvacrol in the digestive tract of chickens to confirm that our galenic allowed delayed release of carvacrol in the caeca. The majority of the administered carvacrol was found in the caeca, the colon and the droppings.

The next step of our study will be to test the new formulation on chicken *in vivo*.

Campylobacter species contamination in UK fresh whole raw chickens and associated packaging at retail sale

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Background: Chicken meat has been incriminated as the key food-borne transmission route for *Campylobacter* infection. In 2015, 73% of UK fresh whole raw chickens tested from February 2014 to March 2015 had ≥10cfu/g *Campylobacter* present. This included 19.4% of samples with levels >1000cfu/g. The outer packaging was identified to be contaminated in 6.8 % of samples.

Objective: Determine the trend in levels of *Campylobacter* in neck skin samples from fresh whole raw chickens and on outer packaging at retail sale from July 2015 to 2016.

Methods: Fresh whole raw chickens (n=2998) were purchased from retail sale based on market share data. Outer packaging was swabbed to establish the levels of *Campylobacter* present on the external surface. Levels of *Campylobacter* were enumerated from neck skin as outlined in EC ISO/TS 10272-2 (2006). Detection limits of 10cfu/g and 10cfu/swab were used. At sample collection a range of data was collected, including weight and 'Use By' date.

Results: The proportion of *Campylobacter* in fresh whole chicken at retail in the UK in the survey period was 61.3 %. Also in this time period, 11.4 % of samples had >1000 cfu/g chicken skin.
In 5.5 % of samples *Campylobacter* were detected from the outer packaging swab, with levels of between 100 and 5740 campylobacter cfu per swab detected in 1.2 % of samples.

Conclusions: The UK poultry industry show evidence that levels of *Campylobacter* species are reducing and that interventions are being effective.

Diamond Like Carbon Ag Nanocomposites as a control measure against *Campylobacter jejuni* and *Listeria monocytogenes* on food preparation surfaces

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Campylobacter jejuni and *Listeria monocytogenes* pose a great risk for human health as high numbers of human campylobacteriosis and listeriosis cases are reported each year in European Union. Despite the different resistance to volatile environmental conditions, both *C. jejuni* and *L. monocytogenes* can survive on food preparation surfaces during processing of raw meat, creating a probability for cross-contamination. Therefore, the aim of this study was to evaluate the potential of surfaces covered with diamond like carbon (DLC) nanocomposite with embedded silver nanoparticles (DLC:Ag) as a control measure against *C. jejuni* and *L. monocytogenes* in food preparation places.

DLC:Ag film contained 22 at.% Ag. Ag nanoparticle size measured by transmission electron microscope (TEM) was in 5-10 nm range. DLC based silver nanocomposites were reactive magnetron sputtered on crystalline silicon coupons. *C. jejuni* and *L. monocytogenes* numbers were counted by two different methods: culture-based enumeration on selective agars and quantitative real-time PCR (qPCR) including staining with propidium monoazide (PMA).

Culture-based enumeration revealed that *C. jejuni* numbers were reduced by 4.06 $\log_{10}$CFU/ml after 15 min and 3.61 $\log_{10}$CFU/ml after 30 minutes on DLC based silver nanocomposite coated silicon coupons in comparison to control samples ($P \leq 0.05$). However, no statistically significant differences were determined between *L. monocytogenes* numbers on DLC:Ag coupons and control coupons after 1 and 4 hours of experiment. Nonetheless, *L. monocytogenes* was not detected on DLC:Ag coupons after 24 h of exposure ($P \leq 0.05$).

PMA-qPCR showed that viable *C. jejuni* and *L. monocytogenes* numbers were underestimated when counted by culture-based method. *C. jejuni* and *L. monocytogenes* affected by DLC nanocomposites with embedded Ag nanoparticles expressed a reduced ability to grow on culture media, but maintained viability during the whole experiment. These findings suggest that DLC based Ag nanocomposites induced the formation of viable-but-not-culturable cells of *C. jejuni* and *L. monocytogenes*.

Proficiency testing organised by the European Union Reference Laboratory (EURL) for *Campylobacter* – the work before, during and after

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In July 2006, the European Commission appointed the National Veterinary Institute (SVA) in Sweden to become the European Union Reference Laboratory (EURL) for *Campylobacter*.
The main functions and duties of EURLs are stated in Article 32 of Regulation (EC) No 882/2004 on official controls. The activities at the EURL-*Campylobacter* include the organisation of an annual workshop, evaluation and development of analytical methods, providing scientific and technical assistance to the European Commission and to the National Reference Laboratories (NRLs), and organisation of annual proficiency tests (PTs). In the PTs, the NRLs are evaluated for their ability to detect, quantify and identify *Campylobacter* spp. in different types of matrices.
At the EURL-*Campylobacter,* the work with proficiency tests starts with testing of matrices and bacterial strains. Matrices consist of both food and animal samples and are chosen from farms with limited previous records of *Campylobacter* in the *Campylobacter* monitoring program. Survival and homogeneity of *Campylobacter* strains in the matrices are tested and so are also the production of the tests.
The work with the PTs ends about a year after it was started, with presentations on the annual workshop, with a summarisation of the results in a report sent to the NRLs and DG SANTE, and with assistance to the NRLs that have underperformed.

Assessment of microbiological criteria for *Campylobacter* in poultry carcasses

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The results of the National Monitoring Program on *Campylobacter* contamination in chicken broiler production chain were used to determine the parameters for a simulation model to assess microbiological criteria for *Campylobacter* in poultry carcasses. The Program collected 403 sets of neck skin samples from 5 chicken each and tested them for enumeration *Campylobacter sp*. per gram of skin, according to ISO methods. Each sample was classified as Negative or Positive: Negatives were samples <100/g and Positives were those ≥100/g. The hypothesised criteria were: number of tested samples (n)=50 (pools of 3), and positivity threshold (m)=10^3/g for all criteria and a variable maximum number of samples above the threshold allowed: (c)=5, c=10, c=15, c =20. A preliminary statistical analysis of the collected data was performed: from the log10 transformed distribution, the average and standard deviation of a set of left-truncated normal distributions were calculated using a Monte Carlo Markov Chain with OpenBUGS. Then, a hierarchical simulation model was designed to simulate the overall positivity of the batch firstly, and then the positivity of each sample in the batch, by using a Bernoulli distribution, with a Beta distributed uncertainty. Finally, the concentration of Campylobacter in the positive samples was extracted from the left-truncated normal distribution with parameters produced as the output of the analysis of the results of the Program. Each simulated batch was compared with the three microbiological criteria, and the expected frequency of compliance and non-compliance with each of the criteria were calculated. The probabilities to get a positive result for the three hypothesized criteria were 7.676% (c=5), 0.142% (c=10), 0.000% (c=15), and 0.000% (c=20) respectively. These results can be used by the competent authority to program a progression of increasingly stricter measures to make the control of Campylobacter more economically affordable.